UNIVERSITY OF LONDON
GALTON LABORATORY, UNIVERSITY COLLEGE

EUGENICS LABORATORY MEMOIRS, XXXVII

AN INTRODUCTION TO HUMAN BIOCHEMICAL GENETICS

H. HARRIS
M.A., M.D.

WITH A FOREWORD BY
L. S. PENROSE
M.D., F.R.S.

WITH A FRONTISPIECE
AND 33 TEXT-FIGURES

PUBLISHED FOR THE
GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON
BY THE
CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22

1953

Price: 15*s. net*

ANNALS OF EUGENICS

A JOURNAL OF HUMAN GENETICS

EDITED BY

L. S. PENROSE

WITH THE ASSISTANCE OF

JULIA BELL	MARY N. KARN
R. A. FISHER	R. R. RACE
J. B. S. HALDANE	J. A. F. ROBERTS

A volume of the *Annals of Eugenics* will in normal times contain about 400 pages with plates and tables. An endeavour will be made to issue a volume annually. Papers for publication should be sent to the Editor. It is a condition of publication in the *Annals* that the paper shall not already have been issued elsewhere and that it will not be reprinted without leave of the Editor. Diagrams and drawings should be sent in a state suitable for photographic reproduction. Papers can be accepted in French, Italian or German. In the last case the manuscript should be in Roman, not German characters.

The *Annals of Eugenics* publishes bodies of original data *in extenso*, in order to make them available for future study. A summary statement bringing out the important features of the data may, however, take the place of original observations, too extensive to be published in full, provided that these are filed in the archives set up for their reception at the British Museum (Natural History).

Contributors receive 25 copies of their papers free. Orders for additional copies should be sent when the proofs are returned.

The distribution of the Journal is in the hands of the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W.1. All orders and enquiries should in future be sent to this address, either direct or through any bookseller or agent. In the U.S.A. the Journal will be handled by the American office of the Press at 32 East 57th Street, New York, 22.

Subscription price for current volume of four parts: 57*s.* net *post free.* Single parts: 15*s.* net plus postage. Back volumes: Vols. 1–5, four parts (in two Double Numbers), 70*s.* net per volume, plus postage. Vols. 6–17, four parts, 60*s.* net per volume, plus postage. Index to the first fourteen volumes of the Journal can be supplied on request, price 5*s.* net plus postage.

Published for the

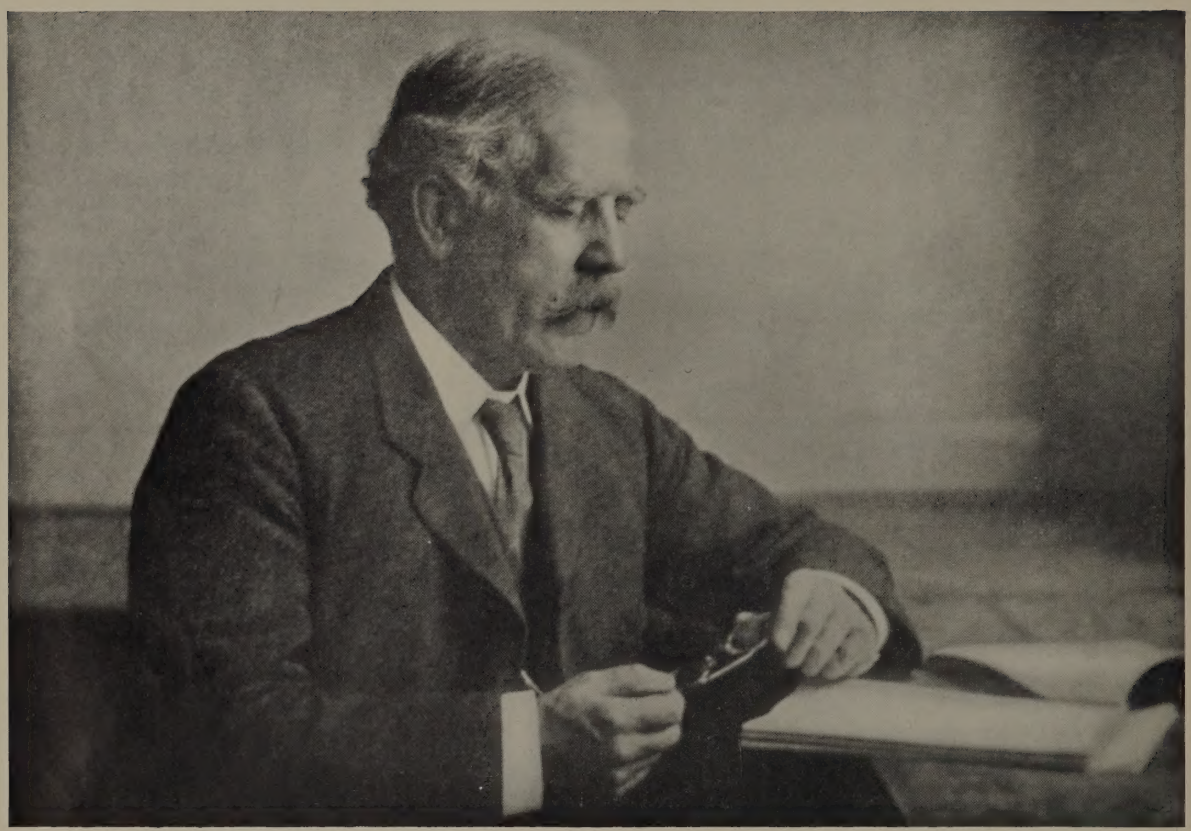
GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON

by the

CAMBRIDGE UNIVERSITY PRESS

London: Bentley House, N.W.1

New York: 32 East 57th Street, 22



A. E. GARROD 1858–1936

UNIVERSITY OF LONDON
GALTON LABORATORY, UNIVERSITY COLLEGE

EUGENICS LABORATORY MEMOIRS, XXXVII

AN INTRODUCTION TO HUMAN BIOCHEMICAL GENETICS

H. HARRIS
M.A., M.D.

WITH A FOREWORD BY
L. S. PENROSE
M.D., F.R.S.

WITH A FRONTISPIECE
AND 33 TEXT-FIGURES

PUBLISHED FOR THE
GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON
BY THE
CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22

1953

The author would like to thank Dr C. E. Dent for the original photographs reproduced in Fig. 3 and Fig. 17; the editor of *Lancet* for permission to reproduce Fig. 5; The Johns Hopkins Press for permission to reproduce Fig. 18; Grune and Stratton Inc. the publishers, and Dr Damashek the editor of *Blood* for permission to reproduce Figs. 19, 23 and 25; The American Association for the Advancement of Science for permission to reproduce Fig. 21; the editor of *Nature* for permission to reproduce Fig. 22; Dr J. V. Neel for permission to reproduce Fig. 24; Dr Grethe Hartmann for permission to reproduce Fig. 26, also St Bartholomew's Hospital for permission to reproduce the photograph in the Frontispiece.

He would also like to thank Professor J. B. S. Haldane, Professor L. S. Penrose, Professor F. L. Warren, Dr C. E. Dent and Dr E. M. Crook for much helpful criticism and advice.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)

CONTENTS

FOREWORD

The scientific study of inborn biochemical peculiarities was initiated by the work of Garrod (1902). His fundamental contribution on alkaptonuria showed clearly how a constitutional metabolic disturbance could be caused by the presence of a single unusual hereditary unit, in this case by a recessive gene in homozygous form. The contributions made to this branch of human genetics were later on summarized by Garrod (1923). Since then the subject has developed rapidly, especially during the last decade. The advances have been due partly to the discovery of diseases whose biochemical basis was previously unsuspected, as in imbecility associated with phenylketonuria and anaemia associated with the sickle-cell phenomenon. Human investigations have also been greatly stimulated by the parallel and much more extensive experimental researches carried out, for example, on pigments of flowers and upon biochemical mutants of *neurospora* and *aspergillium*.

Formerly, the subject of human biochemical genetics could be dismissed as being concerned only with a few rare diseases. Every individual, however, differs from nearly every other in genetical structure, and it may be assumed that he differs in a corresponding way from nearly every other in chemical structure, though tests are not yet accurate or subtle enough to demonstrate this. Some of the hereditary metabolic anomalies already known are harmless, like the specific peculiarities underlying taste deficiency or fructosuria. Others, such as the lipoidoses responsible for the amaurotic idiocies, are invariably accompanied by disease. From the point of view of clinical applications, the study of conditions, like cystinuria, which give rise to disease in certain circumstances but not invariably, are most interesting. These investigations foreshadow as yet unexplored therapeutic possibilities, based upon a precise knowledge of individual inborn biochemical constitution, in relatively common diseases.

In the present monograph, Dr H. Harris brings human biochemical genetics up to date by including in his description the knowledge which has accumulated since the time of Garrod. The field has not been exhaustively covered, but a very great number of aspects have been treated in detail. The necessity is emphasized of understanding precisely the biochemical mechanisms which are now being illuminated rather than of speculating upon obscure processes. Thus, the bibliography, though it contains the essential references, is not intended to be complete. Moreover, certain branches of the subject, for example the chemistry of haemoglobins and of the blood-group substances, are developing so rapidly that by the time this memoir is published some important new additions to knowledge will probably have been made.

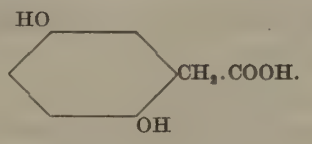
Dr Harris's treatment is confined to those human metabolic traits which have been most thoroughly investigated. Certain conditions, albinism, porphyria and the lipoidoses, have not been considered here because the present state of knowledge about them is confused and not susceptible to convincing analysis. The result is that Dr Harris has been able to present an extremely clear and fascinating account of the essential features of biochemical genetics in man, indicating its clinical implications and the probable lines of its future development.

L. S. PENROSE

I. INBORN BIOCHEMICAL VARIATION

Human beings vary in the details of their metabolic processes and of their biochemical structures. Such differences are very often inborn, and it was Sir A. E. Garrod, a distinguished English physician, who, in his classical paper (1902) entitled 'The incidence of alkaptonuria: a study of chemical individuality', first directed attention to the biological significance of variations of this kind.

Alkaptonuria is a rare condition characterized by the constant daily excretion in the urine of several grammes of homogentisic acid



The urine, when freshly passed, shows little abnormality of colour, but, in contact with air, it soon begins to darken. Alkalinity speeds up the change and the urine passes through various shades of brown and finally appears quite black. Linen and woollen fabrics moistened with the urine become darkly stained and, as a result, many instances of the condition have been recognized in early infancy by the characteristic staining of the napkins. The substance is excreted continuously throughout life. In other respects, the individuals concerned appear to be quite healthy, except that, as they grow older, their cartilages tend to become darkened, and they are somewhat more liable than other people to develop osteoarthritis.

Garrod made several fundamental points about the condition. He observed that a person is either frankly alkaptonuric or conforms to the normal type. That is, he either excretes several grammes of homogentisic acid daily, or none at all. The homogentisic acid was no doubt derived from the amino-acids tyrosine and phenylalanine, and the essential peculiarity of such individuals was their inability to break down in metabolism the benzene rings of the aromatic amino-acids. Instead, they passed them intact. In view of the virtual harmlessness of the peculiarity it could hardly be regarded in the ordinary sense as a disease, but had to be considered as a normal variant, an alternative course of metabolism.

The other striking feature of this condition to which he drew attention was its familial distribution. It often occurred among several members of the same family. Frequently two or more of a group of brothers and sisters would be affected, the parents usually being quite normal as were the more distant relatives. Furthermore, it had been noticed that parents of alkaptonurics were often blood-relatives, generally first or second cousins. This familial distribution makes a highly characteristic pattern, and Garrod had no hesitation in concluding that it implied an hereditary or genetical basis for the condition. It was possible to take this conclusion further. Bateson, whom Garrod consulted, was able to point out that such a familial distribution could be readily explained in terms of the then recently rediscovered Laws of Mendel. The frequent occurrence of the disorder among the brothers and sisters of an alkaptonuric, its rarity among their antecedents, and the high incidence of consanguineous marriage among the parents, who themselves were unaffected, was precisely the type of familial concentration to be expected on the hypothesis that alkaptonuria was inherited as a typical Mendelian recessive character.

Garrod thus interpreted alkaptonuria as an inborn metabolic variant determined by a single recessive Mendelian factor. The metabolic anomaly lay in the peculiar inability of such individuals to break down further the benzene rings of the aromatic amino-acids. He considered that this kind of genetically determined biochemical variation was not a unique phenomenon, but was probably of general occurrence, and that in due course many other examples of such chemical individuality would come to light. In his Croonian lectures (1908) and in his book, *Inborn Errors of Metabolism* (1923), he developed this argument, illustrating it with such conditions as cystinuria, pentosuria, porphyria and albinism.

Since that time many other disorders which fall into the same class of metabolic variants have been studied. Typical examples are phenylketonuria, fructosuria, glycogen storage disease, methaemoglobinaemia and galactosuria. Although they represent a diverse series of biochemical peculiarities, nevertheless they have in common certain characteristic features which make it profitable to study them together.

Characteristically they each serve to divide human beings more or less sharply into two distinct groups differing metabolically in one or another respect. Garrod pointed out that an individual is either frankly alkaptonuric or he is not. The same is true, for example, of pentosuria. This is a condition characterized by the daily excretion of one or more grammes of L-xyloketose in the urine. The amount of this pentose excreted by any one individual is influenced hardly at all by the variations in diet (Enklewitz & Lasker, 1933; Margolis, 1929). Such individuals are thus sharply differentiated from normal persons who do not excrete any L-xyloketose at all in the urine. Other than by the occurrence of this abnormal metabolite in the urine, these people cannot be differentiated from normal individuals, because the condition appears to be harmless and completely asymptomatic. Another striking example of the division of human beings into two sharply differentiated groups, on the basis of their biochemical make up, is provided by the condition known as phenylketonuria. Here the outstanding difference lies in the high concentration of the amino-acid L-phenylalanine in the body fluids compared with that usually found. Thus, in the plasma the phenylalanine concentration is some twenty to thirty times greater than that occurring in normal individuals, though the concentration of the other amino-acids appears to be much the same as in other people. In both pentosuria and phenylketonuria the differentiation from the normal type may be recognized at any time and under any environmental conditions. However, this is not an essential feature. There are analogous disorders where, although the difference in biochemical organization may be just as sharply demarcated from the normal, it can only be recognized in certain environmental situations. Fructosuria is a disorder in which there is an inability on the part of the organism to utilize fructose completely, whether this sugar is ingested either in its simple form or as a sucrose. In such individuals some 15 % of all the fructose taken in the food is excreted in the urine. If, however, the person is fasting or is on a diet free from fructose, no sugar appears in the urine and the condition cannot be detected. Galactosuria is a comparable condition in which there appears to be an inability to metabolize galactose (Mason & Turner, 1935). On a milk-free diet these children appear to be quite normal. When fed with milk their blood galactose level rises, they excrete galactose in their urine, and they become very ill. They lose weight, there is an enlargement of the liver, and they become jaundiced. After a few months cataract may develop.

The underlying biochemical peculiarity in all these conditions appears to persist relatively unchanged and with little intrinsic variation throughout the life of the individual concerned.

Cases of fructosuria have been observed over periods of twenty to thirty years and have shown no change in their fundamental features. Alkaptonurics have frequently been described in whom the condition was first recognized in infancy, as a result of the characteristic staining of the napkins, and has been known to persist unchanged throughout their lives.

Methaemoglobinaemia is an example of a disorder which may readily respond to specific treatment, an apparently complete 'cure' being obtained. That the underlying peculiarity persists, however, is indicated by the immediate relapse resultant on the cessation of therapy. In this condition, a large proportion of the haemoglobin in the red cells exists as methaemoglobin, that is, pigment in which the haemoglobin iron is in the ferric state and is incapable of carrying oxygen. As a result the patients exhibit a characteristic slaty blue colour, which is generally recognized early in life, and, in the absence of treatment, persists with very little variation. Administration of vitamin C (in large doses) or of methylene blue leads to reduction of the methaemoglobin and removes the cyanosis. If the treatment stops the patient rapidly reverts to his previous state. It is reasonable, then, to regard such biochemical peculiarities as representing a distinct aspect of the particular person's individuality.

All these conditions occur much more frequently among the close relatives of affected individuals than in the general population. Thus Munro (1947) found, among 179 brothers and sisters of his phenylketonuric patients, thirty-eight further cases of phenylketonuria. He estimated that, in the general population from which they were drawn, the frequency of the condition was of the order of 1 in 40,000. Lasker (1941) found three cases of fructosuria among eleven brothers and sisters of five fructosuric individuals. Yet fructosuria probably occurs in only one individual in every 500,000 in the general population. Similar evidence is available for most of the other conditions mentioned. Now, since these disorders are in general little, if at all, susceptible to ordinary environmental changes, the familial concentration of cases which is observed can hardly be accounted for in terms of the differences in environment under which different family groups may live. That the conditions are genetically determined is the simplest explanation of the high familial incidence. The occurrence in some cases of an increased incidence of parental consanguinity and the existence of highly characteristic types of pedigree configurations afford further evidence for the view that the conditions are hereditary and inborn.

The high degree of biochemical specificity involved affords another point of interest. A person may be unable to metabolize fructose completely, but the metabolism of glucose and other sugars is not abnormal. His brothers and sisters are more likely than an unrelated person to exhibit fructosuria. They are, however, no more likely than anybody else to manifest some other quite different metabolic peculiarity. Quite closely related disorders, such as phenylketonuria and alkaptonuria, both of which represent abnormalities in the oxidation of the aromatic amino-acids, occur independently and are inherited quite specifically.

Since these peculiarities are inherited their effects on the viability of the individual affected and on his capacity to reproduce are of some biological significance. There are considerable differences in these respects. Fructosuria and pentosuria appear to confer no disadvantage at all on affected individuals. They are as healthy as other people and there is no obvious curtailment of reproductive ability. Alkaptonurics are similarly hardly at all incapacitated by their disorder, although it is true, that, in later life, they are more prone than the average person to develop osteoarthritic changes. On the other hand, a condition such as galactosuria, while being an inborn metabolic disorder of the same type, is a severely crippling disease and, if left

untreated, leads to death in childhood. Phenylketonuria, while not necessarily leading to an early death, is, nevertheless, always associated with a severe degree of intellectual defect, amounting in most cases to idiocy or imbecility. There is a drastic reduction in biological fitness as the patients are nearly always infertile. Between the two extremes of lethality and harmlessness all degrees of impairment of health and fitness may be encountered. Cystinurics are sometimes perfectly healthy and suffer no inconvenience in spite of the fact that they may be passing $\frac{1}{2}$–1 g. of cystine daily in their urine. Often, however, the high concentration of the urinary cystine leads to the formation of stones in the renal pelvis and severe disability may result in consequence. Methaemoglobinaemia, apart from the peculiar cyanotic appearance to which it gives rise appears in most of the affected cases to lead to no ill effects. Sometimes there is a moderate degree of dyspnoea on effort and consequently a restriction on physical activity, and occasionally, as in the case described by Hitzenberger (1933), the disorder may be associated with dwarfing and mental defect. It is thus difficult to draw any sharp line between what may be regarded as normal variants and what are to be considered as pathological. Every degree of intermediacy may be found.

Thus the group of conditions which Garrod called 'inborn errors of metabolism' can be regarded as biochemical variations which sharply characterize individual human beings. They are genetically determined, highly specific, and represent a diverse series of metabolic phenomena. Their influence on the viability of the organism and on its biological fitness, that is, its ability to reproduce, is very varied. Garrod (1902) summarized his position in the following terms:

> If it be, indeed, the case that in alkaptonuria and the other conditions mentioned we are dealing with individualities of metabolism and not with the results of morbid processes the thought naturally presents itself that these are merely extreme examples of variations of chemical behaviour which are probably everywhere present in minor degrees and that just as no two individuals of a species are ever absolutely identical in bodily structure neither are their chemical processes carried out on exactly the same lines. Such chemical differences will obviously be far more subtle than those of form, for whereas the latter are evident to any careful observer the former will only be revealed by elaborate chemical methods.

The conditions discussed by Garrod were all rare. The demonstration that subtle chemical differences between human beings are in fact a common phenomenon was provided by Landsteiner's fundamental work on the human blood groups, the first results of which were published about the same time (1901). He found that when blood serum from one individual is mixed with red cells from other individuals clear-cut individual differences are found. In certain cases marked agglutination of the red cells occurs. In others the red cells are unaffected. On the basis of cross-agglutination tests it became possible to differentiate four classes of people, according to whether they possessed on their red cells one, both or neither of two antigenic substances which became known as *A* and *B*. The serum of an individual does not contain antibodies to the antigens present in his own red cells. It does contain antibodies reacting specifically to the antigen or antigens not present. Thus the four classes of people may be specified in the following way:

Blood group	Antigens on red blood cells	Antibodies in blood serum
O	.	Anti-*A* and anti-*B*
A	*A*	Anti-*B*
B	*B*	Anti-*A*
AB	*A* and *B*	.

In the United Kingdom, approximately 46·7 % of people are group *O*, 41·7 % group *A*, 8·6 % group *B*, and 3·0 % group *AB*.

It is now known that the antigens are mucoids made up of a polysaccharide component associated with an amino-acid complex. The antibodies are proteins belonging to the serum γ-globulins. As yet, the different substances producing the group specificity can only be identified by the characteristic nature of their serological reactions.

Since the red blood cells are being continuously destroyed and new ones formed, and since the serum proteins are probably in a similar state of dynamic equilibrium, it is likely that the specific substances are being continuously synthesized by the individual throughout life. The specific group differences between the four classes of individuals therefore imply subtle differences in the character of their synthetic processes.

During the last fifty years a number of other systems of blood-group antigens which are independent of the *ABO* system have been discovered. In general, they differ from the classical *ABO* blood-groups by the occurrence in most individuals of only the specific antigens and not of the reciprocal antibodies. By use of all the known antisera for the various types of antigens it is theoretically possible to define more than a million different classes of individual according to whether they possess one or other combination of the different antigens (Race & Sanger, 1950). Many such combinations would of course be extremely rare. The discriminative power of these techniques is, however, illustrated by the findings of Race and his colleagues (Bertinshaw, Lawler, Holt, Kirman & Race, 1950) who, using seventeen different antisera, were able to classify 475 Londoners into 296 distinct blood-group combinations. Of these 211 occurred once, forty-five twice, seventeen three times, nine four times, seven five times, one six times, four seven times, one eight times, and one ten times. This then affords a measure of the individual differentiation occurring in respect of one relatively minor feature of the body's biochemical architecture.

II. MENDELIAN HEREDITY IN MAN

In most animals and plants knowledge of the genetical basis of particular inborn differences is derived from the analysis of deliberate and often highly elaborate breeding experiments. In human beings this kind of evidence is not available. We are left only with the possibility of inferring the nature of the genetical processes involved by considering the manner in which particular characters, distinguishable by one or more techniques, are distributed in human populations, and, more particularly, within family groups.

Before we can consider the kind of argument used in the genetical analysis of human data, the definition of certain terms in constant use is necessary.

CHROMOSOMES AND GENES

An individual arises from the fusion of two cells or gametes derived from each of his parents: the ovum from his mother, the sperm from his father. The hereditary potentialities of the zygote formed by the fertilization of the ovum by the sperm are derived from the characteristics of the two component cells. The particular material which appears to be concerned with most of the genetical character of the cells is nucleoprotein organized in the nucleus in the form of a series of structures known as chromosomes. In man there are twenty-four such chromosomes in each gamete, and the resulting zygote contains forty-eight (made of twenty-four homologous pairs). At each cell division or mitosis these divide in such a way that each cell subsequently formed in the developing individual contains the same number of these structures. The main exception to this arises in the formation of the sex cells or gametes of the new individual. Here a special type of cell division occurs (meiosis) resulting in only one of each pair of homologous chromosomes appearing in each gamete, and the total complement of chromosomes present in such cells therefore is twenty-four. (23)

Each chromosome appears to be differentiated longitudinally into more or less discrete regions with genetically distinct properties, these chromosomal subdivisions are known as the genes, and represent the specific units involved in Mendelian heredity. They are self-duplicating units, that is to say each gene is capable of catalysing the production of a unit or units exactly like itself. At cell division each daughter cell comes to contain a gene of the same type as the parent cell. It is not clear whether one of these two is to be regarded as the original gene and the other as a copy, or whether the original gene goes and two replicas are produced. This process of self-duplication is a very exact one, and only very occasionally does a gene fail to reproduce its like. The production from one gene of a gene having somewhat different properties is called a mutation and may occur with a frequency of the order of 1 in 10^7 or 10^8 divisions.

The position of a gene on a particular chromosome is called its locus. A gene at any one locus may, as a result of past mutations, exist in one or more alternative or allelomorphic forms. In general, not more than two allelomorphs of a particular gene can exist in one individual, each occupying the same locus in the two homologous chromosomes, and one being derived from each parent. If the two genes at one locus are the same the individual is said to be homozygous with respect to that particular gene, if they are different he is heterozygous.

Current genetical thought would regard each gene as being responsible for a unit biochemical

process, its status in the economy of the cell being comparable with that of an organ in the body. Genes at different loci will, in general, be concerned with different unit processes (Haldane, 1950).

If one considers the simple situation where, in a population of individuals, there may exist at a particular chromosomal locus one or other of two allelomorphic genes **A** and **a**, then three types of genetically distinct individuals may occur, and their genotypes can be represented as **AA**, **Aa** and **aa**. Our knowledge of such a gene difference depends on our ability to distinguish one or more of such types of individual. Occasionally our techniques of investigation are such that all three types may be clearly distinguished each from the others. More often only two classes of individual can be identified: the one which we can designate as *a* corresponding to the genotype **aa** and the other *A* corresponding to the genotypes **Aa** and **AA** which are indistinguishable. In such circumstances we say that the gene **A** is 'dominant' to **a**, and the gene **a** 'recessive' to the gene **A**. The class *A* is called the phenotype corresponding to the genotypes **Aa** and **AA**.

HEREDITY OF THE *MN* BLOOD GROUPS

The most direct demonstration of Mendelian inheritance in man has been furnished by the study of the blood-group antigens. The *MN* groups provide a typical example. Landsteiner & Levine (1928) were able to prepare in rabbits, by the injection of red cells from different human beings, two antisera which were specific for two different antigenic substances occurring in human red cells. People could be divided into three classes according to whether their red cells were agglutinated with one, the other, or both of the sera. The antigens were called *M* and *N* and the antisera anti-*M* and anti-*N*. The authors put forward the hypothesis that these differences were produced by two allelic genes of approximately equal frequency, one of which determined the synthesis of the antigen *M* and the other that of the antigen *N*. The three different phenotypes and genotypes on this hypothesis can be represented as follows:

Phenotype	Response with		Genotypes
	Anti-*M*	Anti-*N*	
M	+	−	**MM**
MN	+	+	**MN**
N	−	+	**NN**

On such a hypothesis the following distribution of children among the offspring of different types of matings would be expected:

Parental combination	Percentage of children of different types		
	M	*MN*	*N*
M × *M*	100	.	.
M × *N*	.	100	.
N × *N*	.	.	100
MN × *M*	50	50	.
MN × *N*	.	50	50
MN × *MN*	25	50	25

Extensive family studies have shown very good agreement with these theoretical requirements. The largest body of data is that collected by Wiener (1951) and his collaborators over the last twenty years. The results are given in Table 1. It will be seen that, out of 2734 children there were only six exceptions to the results expected on the theory of Landsteiner & Levine. It is believed that these can be accounted for as being due to illegitimacy.

TABLE 1. *Distribution of MN blood groups in* 1160 *families.* (*Percentages in brackets*)

After Wiener (1951).

Parental combination	Number of families	Children belonging to type						Totals
		M		*MN*		*N*		
$M \times M$	119	272	(99·6)	1	(0·4)	0	(0·0)	273
$M \times N$	142	1	(0·3)	315	(99·7)	0	(0·0)	316
$N \times N$	33	0	(0·0)	0	(0·0)	72	(100·0)	72
$MN \times M$	341	408	(51·3 ± 1·2)	387	(48·6 ± 1·2)	1	(0·1)	796
$MN \times N$	250	3	(0·5)	334	(52·4 ± 1·3)	300	(47·1 ± 1·3)	637
$MN \times MN$	275	163	(25·1 ± 1·2)	317	(49·5 ± 1·3)	160	(25·0 ± 1·2)	640

RECESSIVE CHARACTERS

When a character is observed in only a small proportion of the population elucidation of the genetical situation is more complex. Nevertheless, it is frequently possible by examining the manner in which the character is distributed in families, each of which contains at least one affected member, to arrive at reasonably firm conclusions as to the nature of the underlying genetical processes.

This was first appreciated by Garrod and Bateson, who pointed out that the highly characteristic familial pattern observed in alkaptonuria could readily be understood on the hypothesis that the condition was inherited as a typical Mendelian recessive character. A rare disorder inherited in this way would be expected to show two very characteristic features. In the first place, it should occur relatively frequently among the brothers and sisters of affected individuals, and only rarely among their parents, children or more remote relatives. Secondly, the parents of such patients would quite often be found to be first or second cousins. The overall incidence of consanguineous marriages among the parents of these cases should be higher than that encountered in the general population. The theoretical reasons for this conclusion are of importance and involve the concept of gene frequency.

Consider a population of individuals in whom at any particular chromosomal locus there may occur either the gene **A** or the gene **a**. As every chromosome is represented twice in each person, there will be three types of individual found in such a population—**AA**, **Aa** and **aa**. If we assume that the population is mating at random then the relative frequencies with which the three genotypes will be found is given by the following formulae (Hardy, 1908; Weinberg, 1908):

$$\mathbf{AA} : \mathbf{Aa} : \mathbf{aa}$$
$$p^2 : 2pq : q^2$$

where p = frequency of the gene **A** in the population, q = frequency of the gene **a** in the population, and $(p+q)=1$. Nine different types of mating may occur in such a population and their

relative frequencies, and the frequencies of the different types of offspring to which they can give rise, may be readily calculated (Table 2).

TABLE 2. *Frequencies of parental mating types and their offspring, for an autosomal gene pair occurring at random*

Frequency	Parental genotypes	Offspring		
	Father Mother	AA	Aa	aa
p^4	AA × AA	p^4	.	.
$2p^3q$	AA × Aa	p^3q	p^3q	.
p^2q^2	AA × aa	.	p^2q^2	.
$2p^3q$	Aa × AA	p^3q	p^3q	.
$4p^2q^2$	Aa × Aa	p^2q^2	$2p^2q^2$	p^2q^2
$2pq^3$	Aa × aa	.	pq^3	pq^3
p^2q^2	aa × AA	.	p^2q^2	.
$2pq^3$	aa × Aa	.	pq^3	pq^3
q^4	aa × aa	.	.	q^4
	All matings			

If we suppose that individuals suffering from a particular condition are homozygous for the gene **a** which is recessive to **A**, then individuals of the genotype **aa** will show the disorder, and those of the types **Aa** and **AA** will appear normal. Affected individuals (**aa**) can only arise as offspring from the following types of mating:

Father		Mother
Aa	×	**Aa**
Aa	×	**aa**
aa	×	**Aa**
aa	×	**aa**

If the condition is rare, the mating **Aa** × **Aa** will be very much commoner than any of the others, and so the vast majority of cases will be derived from matings of apparently normal individuals. Thus, if the condition occurred in 1 per 40,000 members of the population, the frequency of the heterozygotes, **Aa**, would be 1/100, and the relative frequencies of the four types of mating from which affected individuals could arise would be

Father		Mother	
Aa	×	**Aa**	1
Aa	×	**aa**	1/400
aa	×	**Aa**	1/400
aa	×	**aa**	1/160,000

The chance that an affected individual **aa** would marry another of the same genotype, or a heterozygote **Aa**, is small compared with the chance that he would marry an individual of type **AA**. Thus it is unlikely that he himself would have affected offspring.

On the other hand, the condition will occur not infrequently among the brothers and sisters of an affected individual. They are derived from the same mating (**Aa** × **Aa**) and so approximately 1 in 4 may be expected to be similarly affected. Thus a condition determined by a rare recessive gene will be found frequently among the sibs, but rarely among the parents or children of affected individuals.

The assumption of completely random mating is rarely entirely justified in human populations. There is usually a certain amount of inbreeding, represented by a significantly higher frequency of cousin marriage than would be expected in a random mating system.

The close relatives, i.e. parents, uncles, aunts and cousins, of a heterozygous individual **Aa** are rather more likely to carry the abnormal gene **a**, than is an individual chosen at random from the general population. Thus, if such a heterozygous person marries his first cousin, or some other close relative, the probability that his partner will also be heterozygous is relatively high, and hence the probability that one or more of his offspring will be an affected individual of genotype **aa**.

Consequently, the incidence of cousin marriage among the parents of affected individuals may be expected to be higher than that in the general population. Lenz's (1919) formula

$$F = \alpha/(\alpha + 16q),$$

where α = incidence of first-cousin marriage in the general population and q = gene frequency of the rare recessive gene **a**, gives an approximate estimate, F, of the incidence of cousin marriage to be expected among the parents of individuals homozygous for a rare recessive gene, **a**. It may be derived in the following way. If we consider a heterozygous (**Aa**) individual M, the chance that he will marry his first cousin is α, and the chance that his first cousin is also heterozygous for the gene **a** is 1/8. Thus, the chance that M mates with a heterozygous first cousin is $\alpha/8$. The chance that he mates with an unrelated person is $(1-\alpha)$, and the chance that such a person is heterozygous for **a** is $2pq$. Hence the frequency, F, of first-cousin unions among all matings giving rise to affected homozygous offspring is

$$(\alpha/8)/[\alpha/8 + 2pq(1-\alpha)],$$

and, since both p and $(1-\alpha)$ are very nearly unity, this may, for practical purposes be written

$$\alpha/(\alpha + 16q).$$

Estimates for F, assuming different values of α and of q are given in Table 3. It is apparent that the less frequent is the condition in the general population, the higher is the incidence of cousin marriage to be expected. For similar reasons the occurrence of a parent and child both affected is much more likely to be found if the parent has married a close relative than if he has not.

TABLE 3. *Percentage of first-cousin parentage* (F) *for rare recessive characters*

Case frequency	Gene frequency	Frequency of first-cousin matings in general population (α)		
		0·5 %	1·0 %	2·0 %
q^2	q	(F)	(F)	(F)
1/1000	1/32	1·0	2·0	3·8
1/10,000	1/100	3·0	5·9	11·1
1/100,000	1/316	9·0	16·5	28·3
1/1,000,000	1/1000	23·8	38·4	55·6

That the observed familial distribution and population frequency of a condition may on occasion fit these theoretical requirements very exactly is indicated by the work of Jervis (1939), Fölling, Mohr & Ruud (1945), and Munro (1947) on phenylketonuria. Each of these workers

studied a large series of phenylketonuric patients and obtained accurate data on the occurrence of the disorder among their close relatives. Their main findings are summarized in Table 4. The condition was common among the sibs of the patients but rare among their parents and more remote relatives. The incidence of first-cousin marriage among the parents in the different series varied between 5 and 14 % which is considerably in excess of that generally occurring among the populations from which the cases were drawn.

TABLE 4. *Familial distribution of phenylketonuria*

Source	Initial cases Phenyl-ketonuric	Brothers and sisters		Parents		
		Phenyl-ketonuric	Normal	Phenyl-ketonuric	Normal	First cousins
Munro (1947)	47	38	141	0	94	10
Jervis (1939)	125	72	270	2	248	14
Fölling *et al.* (1945)	22	18	86	0	44	6
Total	194	128	497	2	386	30

It was possible to show that the observed incidence of cousin marriage corresponded quite well with that expected on the Lenz formula. To do this it was necessary to estimate the frequency of the disease in the populations concerned. Munro, working in the United Kingdom, found thirty-one cases of phenylketonuria among 4090 mental defectives of all grades in the mental-defective institutions in which the study was carried out, and Lewis (1929) estimated the incidence of all types of mental defect in the general population as 0·8 %. From these figures the incidence of phenylketonuria should be about six per 100,000 (0·006 %). This estimate, however, is probably too high, because the proportion of patients with severe mental defect to those with mild defect is much higher in institutions than in the outside population. Lewis's estimate of the incidence of imbeciles and idiots in the general population was 0·17 %, and Munro found thirty phenylketonurics among 2457 institutional imbeciles and idiots. On this basis the incidence of phenylketonuria would be two per 100,000 (0·002 %). This figure, however, does not include those phenylketonurics having a milder degree of mental defect. Munro therefore concluded that the incidence of the disease in the general population lies between two and six per 100,000 and is probably nearer the lower figure, perhaps about one in 40,000. This would give a gene frequency of about one in 200 and a frequency of heterozygotes in the general population of about one in 100. If the incidence of first-cousin marriage is taken as 0·008 (Bell, 1940), then the expected frequency of cousin marriage, on the Lenz formula, is given by

$$F = 0{\cdot}008/(0{\cdot}008 + 16 \times 0{\cdot}005) = 0{\cdot}008/0{\cdot}088 = 0{\cdot}09,$$

or about 9 %. This agrees well with the frequency actually found in this survey. Jervis (1939) estimated the frequency of the disease in the United States to be one in 25,000 of the general population, and found a frequency of first-cousin marriage of 5 % which again agrees quite well with the expected values on this hypothesis.

The other direct test that may be applied to such data is to inquire whether the observed proportions of phenylketonuric members to the normal members of the families is consistent with the expected Mendelian ratios. The great majority of cases are derived from normal parents, both of whom are presumably heterozygous (Aa). Consequently, we should expect

one-quarter of their offspring to show the abnormality and three-quarters to be normal. Actually, Munro, in his forty-seven families, found eighty-five cases of phenylketonuria among 226 brothers and sisters derived from normal parents. This gives an incidence of 37·6 %. The reason for this high proportion of affected sibs arises from the method of sampling inherent in the collection of human family data. Because human families are small, many matings of parents, both of whom are heterozygous for the abnormal gene, will give rise to no affected offspring, and so will be left out of any survey of the disease. For every family with a single affected child, there would be three families of single children who are unaffected. In general, for all the sibships of s brothers and sisters derived from heterozygous parents $(\frac{3}{4})^s$ will contain no abnormal children, and $1-(\frac{3}{4})^s$ will contain at least one abnormal child. Only the latter will be recorded in the data, and so the crude proportion of affected to normal children in the families observed will be too high. It is possible to make various corrections for this bias in sampling. One useful one is that recommended by Hogben (1931). For sibships of s members derived from heterozygous parents, the proportion of sibships which might contain a recessive but in fact do not do so is $(\frac{3}{4})^s/[1-(\frac{3}{4})^s]$. If there are n_s observed sibships, the total number which could contain recessive offspring is $n_s+n_s(\frac{3}{4})^s/[1-(\frac{3}{4})^s]=n_s/[1-(\frac{3}{4})^s]$. The total number of individuals corresponding to this is $sn_s/[1-(\frac{3}{4})^s]$, and of these one-quarter should show the expected character, so that the expected number r of affected offspring from fraternities of size s derived from heterozygous parents is given by

$$r=\tfrac{1}{4}\,(sn_s)/[1-(\tfrac{3}{4})^s].$$

Expected and observed numbers may be added up over a series of sibships and compared. Applying this method to Munro's data (Table 5) it is found that a good agreement is obtained between the

TABLE 5. *Factorial method of testing recessive hypothesis on sibships containing cases of phenylketonuria*

After Munro (1947).

Size of sibship (s)	Number of sibships of size S (n_s)	Total number of sibs ($s.n_s$)	Number of sibs phenylketonuric		Variance of expected number* ($n_s.k_s$)
			Observed	Expected $s.n_s/4[1-(\frac{3}{4})^s]$	
1	6	6	6	6·00	0·00
2	7	14	8	8·00	0·86
3	6	18	10	7·78	1·58
4	5	20	8	7·31	2·10
5	7	35	13	11·47	4·14
6	5	30	12	9·12	3·88
7	2	14	4	4·04	1·94
8	3	24	8	6·67	3·52
9	1	9	2	2·43	1·38
10	2	20	5	5·30	3·18
11	1	11	2	2·87	1·81
12	1	12	3	3·10	2·02
13	1	13	4	3·33	2·23
Total	47	226	85	77·42	28·64

* Out of 226 sibs, altogether 85 were affected against 77·42, the expected number on the recessive hypothesis. The difference between the observed and expected values here is 7·58, which is slightly but not significantly greater than the standard error, $\sqrt{28·64}$, or 5·35.

theoretical and expected numbers of abnormal individuals. Several other methods for dealing with the same problem have been suggested (Weinberg, 1927; Fisher, 1934; Haldane, 1938). The manner in which the data are collected, that is to say the way in which each family has been ascertained, has to be taken into consideration in choosing the most appropriate method of calculation.

It is clear that the familial distribution of phenylketonuria satisfies with some precision the requirements of the hypothesis that it is determined by a single recessive Mendelian factor or gene. No alternative hypothesis of equal economy has as yet been advanced to account for this quite characteristic familial pattern.

Phenylketonuria has been studied from the genetical point of view more than most other metabolic disorders. However, there is little doubt that there are many other conditions which are inherited in the same way. Fructosuria is a typical example, but its genetical investigation presents difficulties from two points of view. In the first place, it is completely harmless, so affected individuals do not present themselves to the clinician in the ordinary way. It can only be detected by fairly detailed investigations of the urine of individuals who, for one reason or another, have been found to excrete reducing substances. Furthermore, it is extremely rare. Probably, in European populations, it is ten or more times less frequent than phenylketonuria. This makes the study of a series of cases by any one individual, or even by a group of workers, a matter of considerable difficulty. Lasker (1941) was able to collect five families in which the condition occurred in at least one member of each. The pedigrees she obtained are shown in Fig. 1, and there is no doubt that the condition must be regarded as being determined by an extremely rare recessive gene. The initial cases were found in the first place by the routine examination of urines of individuals who had been found, in insurance examination of urines, to excrete a reducing substance which was not glucose. In a similar way, Lasker, Enklewitz & Lasker (1936) were able to identify a number of cases of L-xyloketosuria and subsequently to study their families. This condition, like fructosuria, is entirely benign although it is probably, at least in some populations, rather more common. Again the distribution of cases in the families corresponded with that expected on the hypothesis of a rare recessive gene.

In many disorders, while there is strong reason to believe that we are dealing with a rare recessive gene as the causative factor in the occurrence of the condition, the evidence is often in one or other respect somewhat incomplete. Fragmentary data arise from the fact that frequently no single observer has been in a position to study a random series of cases and to examine in detail their families. One must depend on the individual reports of families and isolated cases which have at various times, and generally for a variety of different reasons, been recorded in the medical literature. The case may have been described because of the intrinsic interest of the particular patient, because of some particular biochemical investigations that have been conducted, or perhaps because of the striking nature of the familial distribution. In instances where the genetical side of the problem was not being specifically considered it is not surprising that details of the occurrence or non-occurrence of parental consanguinity may have been omitted from the description or that a record of the findings in other members of the family may not be available. When the cases have been recorded for their genetical interest there is a selective tendency for extreme examples with a high familial concentration of the disease to be described as against the less notable instances where perhaps only one affected individual was encountered and all the relatives were normal. A series of cases collected from the literature is therefore inevitably highly selected from one or more points of view, and

consequently caution is required in interpretation. Nevertheless, it is often possible, even in data of this type, to draw fairly firm conclusions concerning the genetical situation involved.

Thus there is no reason to doubt that Garrod's views on the genetics of alkaptonuria, based though they were on quite a small sample of cases, mostly collected from the literature, are substantially correct. His data (1902) have been extended considerably by Hogben, Worrall & Zieve (1932) who reviewed all the cases recorded in the literature up to 1931. In all they found that forty-two isolated cases had been described in which no details of relatives had been given, and forty-five instances where some information about the family was available. The amount

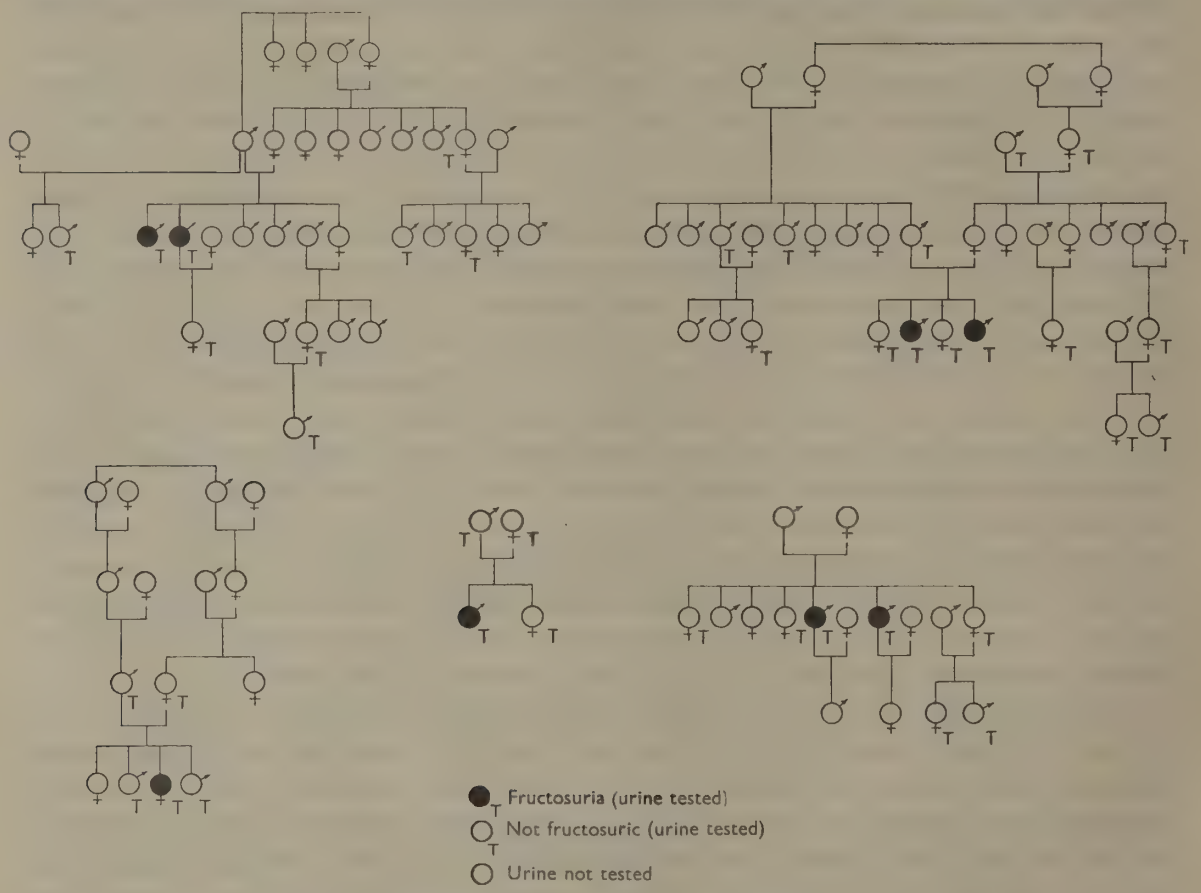


Fig. 1. Pedigrees of fructosuria. (After Lasker, 1941.)

of information varied from case to case. Sometimes it had been definitely stated whether or not the parents were consanguineous; in others no definite statement had been made on this point. In some cases full descriptions of all the close relatives were available, in others this information was only fragmentary. However, a review of this large mass of published material led to some quite unequivocal conclusions. It was clear that, where another member of the family was similarly affected, it was in most cases a brother or sister who was involved and only rarely a parent, child or more remote relative. Furthermore, there was, without doubt, a very high incidence of cousin marriage. In forty-five sibships, involving a total of eighty-three alkaptonurics, thirteen were derived from consanguineous matings, and twenty from parents who were definitely not related. In the other twelve there was no information one way or the

other. If we take into account the other forty-two cases in which no familial information was given at all, we find that, at a most conservative estimate, there were, among 125 alkaptonuric individuals, twenty-seven who were known to be the offspring of consanguineous matings, i.e. a rate of 21 %. The true incidence is probably higher (Table 6).

TABLE 6. *Incidence of parental consanguinity in alkaptonuria*

Relationship of parents	Total alkaptonurics
First cousins	26
Consanguineous (degree unknown)	1
Unrelated	36
Not ascertained	62
Total	125

HETEROZYGOUS OR DOMINANT CONDITIONS

By no means all biochemical abnormalities exhibit a familial distribution of the recessive type. In some cases a completely different situation obtains. Here the disorder is found in several generations of the same family, apparently being transmitted directly from parent to child. One or other parent of each patient is similarly affected. This is the situation, for example, in renal glycosuria, a typical pedigree of which is shown in Fig. 2. Similar families segregating for this condition

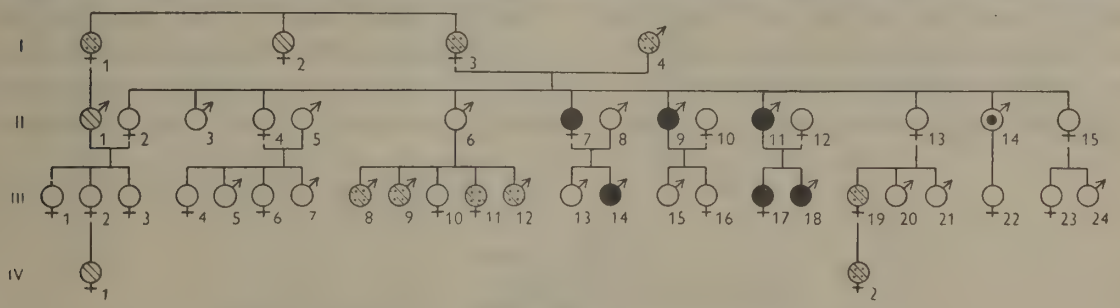


Fig. 2. Pedigree of renal glycosuria. (After Houston & Merivale, 1949.)

have been described by many authors (Hjarne, 1927; Bowcock, 1929: Parkes Weber, 1931), and it is fairly certain that this type of familial configuration is characteristic of the condition.

The simplest explanation is that here we have an anomaly which is manifest in individuals heterozygous for a particular abnormal gene. In such circumstances we would expect to find that one or other of the parents of any particular case was similarly affected, and that approximately half the children also show the disorder. No increase in the frequency of parental consanguinity over that encountered in the general population would be expected. On such a hypothesis, the vast majority of cases of the condition are heterozygotes and the corresponding homozygotes are of necessity relatively rare. Thus, for a heterozygous condition with a case frequency as high as 1 in 200 of the general population, the gene frequency of the abnormal gene $(1-p)$ would be 1 in 400, and the individuals homozygous for the gene would only be expected

to occur with a frequency of 1 in 160,000. They could only arise from the mating of two heterozygous individuals, a markedly rare event, and even then only 1 in 4 of the offspring would be homozygous. In most heterozygous or 'dominant' conditions, therefore, the homozygous gene has not been identified and nothing is known of the degree of severity or even of the kind of disorder to which it would give rise. In this sense the term 'dominant' applied to those conditions is a misnomer as it implies that the heterozygous and homozygous individuals are not distinguishable.

MULTIPLE ALLELES

When more than two allelic genes occur at the same locus, several different genotypic combinations may occur, and the problem of elucidating the genetics of characters determined by such a system of genes may be formidable. The classical example is Bernstein's (1925) demonstration that the blood-group characters *A*, *B*, *AB* and *O* are determined by a series of three allelic genes which may be designated **A**, **B** and **O**.

The earliest theory of the inheritance of these characters was that of van Dungern & Hirszfeld (1910), who suggested that the groups *A* and *B* were determined by two independent systems of two allelic genes at different loci. Thus two gene pairs were postulated, **A** and **a**, **B** and **b**, and, for each allelic pair, three types of individual would be expected, **AA**, **Aa** and **aa**, **BB**, **Bb** and **bb**. **A** was regarded as dominant to **a**, and **B** to **b**. That is to say that, using the two sera, anti-*A* and anti-*B*, all individuals containing at least one **A** gene would be detected by the first serum, and all containing at least one **B** gene would be detected by the second. Where only the alternative genes **a** and **b** were present, as in the double homozygote **aabb**, neither serum would cause agglutination and so the individual would be classified as *O*. The four phenotypic classes observed in the general population would, on this theory, therefore be made up of the following genotypes:

Phenotype	Genotype
O	**aabb**
A	**AAbb, Aabb**
B	**aaBB, aaBb**
AB	**AABB, AaBB, AABb, AaBb**

If the gene frequencies of **a** and **A** are x and $(1-x)$ and of **b** and **B** are y and $(1-y)$, then, in homogeneous populations where there is more or less random mating, the following proportions of the four groups should occur:

Phenotype	Genotype	Frequency	Total frequency
O	**aabb**	x^2y^2	x^2y^2
A	**AAbb**	$(1-x)^2\,y^2$	$(1-x^2)\,y^2$
	Aabb	$2x(1-x)\,y^2$	
B	**aaBB**	$x^2(1-y)^2$	$x^2(1-y^2)$
	aaBb	$2x^2y(1-y)$	
AB	**AABB**	$(1-x)^2\,(1-y)^2$	$(1-x^2)\,(1-y^2)$
	AaBB	$2x(1-x)\,(1-y)^2$	
	AABb	$(1-x)^2\,(1-y)\,2y$	
	AaBb	$2x(1-x)\,2y(1-y)$	

So that the following relationship should hold

(frequency of *O*) (frequency of *AB*) = (frequency of *A*) (frequency of *B*).

Bernstein (1925) pointed out that in fact this relationship failed to satisfy the observed frequencies of the four groups in quite a large number of populations for which data were at that time available. He suggested that the relative frequencies of the blood groups in these different populations were more adequately explained by the hypothesis that three allelic genes (**A**, **B** and **O**) were the genetic determinants. **A** and **B** were regarded as dominant to **O** and so the four blood groups could consist of the following phenotypes:

Phenotype	Genotype	Agglutination with anti-*A*	Agglutination with anti-*B*
AB	**AB**	+	+
A	**AA** and **AO**	+	−
B	**BB** and **BO**	−	+
O	**OO**	−	−

If the gene frequencies of the genes **A**, **B** and **O** are respectively p, q and r where $p+q+r=1$, then the relative proportions of the four classes in a homogeneous population should be

Phenotype	Genotype	Frequency
AB	**AB**	$2pq$
A	**AA**	p^2 } p^2+2pr
	AO	$2pr$ }
B	**BB**	q^2 } q^2+2qr
	BO	$2qr$ }
O	**OO**	r^2

The three gene frequencies are therefore given by

$$r=\sqrt{(\text{frequency of } O)},$$
$$q=1-\sqrt{(\text{frequency of } O+\text{frequency of } A)},$$
$$p=1-\sqrt{(\text{frequency of } O+\text{frequency of } B)}.$$

If the hypothesis is correct the values of p, q and r so obtained should satisfy the equation, $p+q+r=1$. This in fact is found to be the case (Table 7) within the limits of sampling error. By now a very considerable number of different human populations with widely differing relative proportions of the blood-group frequencies have been tested, and the general validity of Bernstein's hypothesis, as far as this aspect of the problem goes, has been fully established.

Of course, the familial distribution of the blood groups must also be adequately accounted for in terms of this theory. The critical families are those in which one parent is *AB*. Among the offspring of such parents no *O* children should be found, and among the offspring of $O\times AB$ parents no *AB* children should occur. Table 8 gives the relevant data derived from Wiener's extensive review of the published material (1943). Thirteen children occur in unexpected groups. This small proportion may be reasonably explained as being due to the combined effects of illegitimacy, technical errors, and conceivably, mutation. According to theory a member of blood group *AB* produces equal numbers of *A* and *B* gametes. The table shows that this is the case, the deviation from equality being less than the standard error of sampling. Finally, in matings $AB\times AB$, the sum of the homozygous offspring *A* and *B* should equal, within the limits of sampling error, the total heterozygotes *AB*. This result is also obtained.

TABLE 7. *ABO blood-group frequencies and the estimated gene frequencies on Bernstein's hypothesis*

After Bernstein (1925), based on data of Hirszfeld & Hirszfeld (1919).

Race	Number of people	Observed frequencies of groups				Theory of von Dungern & Hirszfeld		Theory of Bernstein. Estimated gene frequencies			
		O	*A*	*B*	*AB*	*O*×*AB*	*A*×*B*	*p*	*q*	*r*	*p*+*q*+*r*
English	500	0·464	0·434	0·072	0·031	0·0143	0·0312	0·268	0·052	0·681	1·001
French	500	0·432	0·426	0·112	0·030	0·0129	0·0477	0·262	0·074	0·657	0·993
Italians	500	0·472	0·380	0·110	0·038	0·0179	0·0418	0·237	0·077	0·687	1·001
Serbians	500	0·380	0·418	0·156	0·046	0·0175	0·0642	0·268	0·107	0·516	0·991
Greeks	500	0·382	0·416	0·162	0·040	0·0153	0·0674	0·262	0·107	0·618	0·987
Bulgarians	500	0·390	0·406	0·142	0·062	0·0241	0·0577	0·271	0·108	0·624	1·003
Arabs	500	0·432	0·324	0·190	0·050	0·0218	0·0616	0·209	0·129	0·660	0·998
Turks (Macedonia)	500	0·368	0·380	0·186	0·066	0·0243	0·0707	0·256	0·136	0·607	0·999
Russians	1000	0·407	0·312	0·218	0·063	0·0256	0·0680	0·210	0·152	0·638	1·000
Spanish Jews	500	0·388	0·330	0·232	0·050	0·0194	0·0766	0·213	0·153	0·623	0·989
Madagascans	400	0·458	0·262	0·237	0·045	0·0261	0·0621	0·168	0·154	0·675	0·997
Senegal Negroes	500	0·432	0·224	0·292	0·050	0·0216	0·0654	0·149	0·189	0·657	0·995
Annamese	500	0·420	0·224	0·284	0·072	0·0302	0·0636	0·161	0·198	0·648	1·008
Hindus	1000	0·313	0·190	0·412	0·085	0·0266	0·0783	0·149	0·291	0·560	1·000

TABLE 8. *Familial data on ABO blood groups*

After Wiener (1943).

Parents	Children				Total
	O	*A*	*B*	*AB*	
O×*AB*	8	633	646	3	1290
A×*AB*	0	533	247	312	1092
B×*AB*	2	183	406	232	823
AB×*AB*	0	28	36	65	129

Total *A* gametes 1609; total *B* gametes 1647.

(In the children of the mating *A*×*AB*, the *A* children are derived from *A* gametes of the *AB* parent, and the *B* and *AB* children from the *B* gametes, and so on.)

Total homozygotes from *AB*×*AB* = 64
Total heterozygotes from *AB*×*AB* = 65

III. THE HETEROGENEITY OF APPARENTLY SIMPLE 'CHARACTERS'

One of the problems central to all studies in human genetics arises from the difficulty of knowing whether a particular individual difference has been characterized in, as it were, a 'chemically pure' form. What appears at first sight to be a homogeneous entity readily identifiable by a particular technique, and presumably having a unitary genetical causation, turns out, with the application of newer techniques to the problem, to consist of more than one quite distinct phenomenon.

CYSTINURIA

The condition known as 'cystinuria' provides a simple illustration of this point. It was first recognized by the discovery of a renal calculus of unusual composition (Wollaston, 1810). Similar cases were identified periodically over the next hundred years, and it was found that the stones were nearly entirely composed of the amino-acid cystine. The condition appeared to be hereditary because, although it was evidently uncommon in the general population, more than one member of the same family was not infrequently found to form renal calculi of the same type. Such individuals excreted cystine continuously in large quantities, and cystine crystals were very often to be found in the urinary sediment. It then emerged that individuals could occur who, while appearing to be in perfect health and having no history or signs of stone formation, nevertheless excreted large amounts of cystine daily. They were identified at first by the finding of typical cystine crystals in the urine of normal relatives of patients with cystine calculi. Later this finding was confirmed by the use of simple chemical tests (cyanide-nitroprusside test and Sullivan reaction) for cystine in urine (Lewis, 1932).

More recently, the occurrence of cystinuria in patients with certain rare forms of rickets has attracted attention. As originally described, these patients were invariably infants and the whole syndrome comprised a failure to thrive, often with bouts of vomiting, a severe form of rickets resistant to the usual anti-rachitic doses of vitamin D, chronic acidosis, polyuria, glycosuria of the renal type, and hypophosphataemia (McCune, Mason & Clarke, 1943). This symptom complex is sometimes referred to as cystine rickets, other names are Lignac's disease and the syndrome of Fanconi, de Toni, and Debre. The outlook is in general poor and few cases have survived for many years. Post-mortem examination has often revealed crystals of cystine extensively deposited throughout the body and this phenomenon, known as cystinosis (Abderhalden, 1903), may on occasion be recognized during life by the examination of the bone marrow and of deposits in the cornea (Esser, 1941; Burki, 1941). A similar type of symptom complex, but presenting as a severe osteomalacia in adult life has also been recognized (Stowers & Dent, 1947). In these cases, although cystinuria may occur, cystinosis has not yet been encountered.

It was generally assumed until very recently that all these varieties of clinical abnormality represented different degrees of severity of a single hereditary metabolic disorder whose most characteristic sign was the excretion in large quantities of cystine in the urine.

The application of the technique of partition chromatography on filter-paper to the study of the urinary amino-acids in these cases has led to a complete re-orientation of outlook (Dent,

1947). It has become clear that in these cases cystine is not the only urinary amino-acid excreted in large and abnormal amounts. In a series of cases of the classical type of cystinuria with renal stone formation, Dent & Rose (1951) found that not only cystine but also the basic amino-acids lysine and arginine were present in grossly increased quantities, while the other urinary amino-acids appeared to occur in normal amounts. In cases of patients showing the typical features of Lignac's disease or the Fanconi syndrome an entirely different pattern of urinary amino-acid excretion occurred (Dent, 1947). Here there was a generalized amino-aciduria in which abnormal amounts of serine, glycine, alanine, glutamine, proline, lysine, histidine, arginine, threonine, valine, the leucines, phenylalanine, tyrosine and cystine may be found. The cystinuria previously identified in such cases seems to be merely one facet of a much more generalized disturbance of urinary amino-acid excretion.

In spite of minor individual variations, the two classes of case give quite distinctly different chromatographic results (Fig. 3). There is no difficulty in any one urine of recognizing, by the appearances of the chromatograms, the type to which it belongs. These differences presumably imply distinct differences in the underlying biochemical peculiarities leading to the amino-aciduria. It seemed reasonable, therefore, to regard 'cystinuria' as being heterogeneous and capable of being broken down into at least two distinct entities. The first group would consist of the classical type of case with a tendency to cystine stone formation and the excretion in excessive quantities of cystine, lysine and arginine. The second group comprises those cases in which a chronic generalized amino-aciduria occurred, with cystine also present as one of the amino-acids excreted in excess. It is in this class that we find patients with rickets or osteomalacia resistant to vitamin D, cystine deposits in the tissues, and other features of the Fanconi syndrome. This group does not appear to form stones.

This view was confirmed by the results of family studies (Dent & Harris, 1951). If the close relatives of a series of patients representing the different types of disorder mentioned above are studied further examples of amino-aciduria are found. These may or may not be symptomless. However, among the relatives of the patients with the cystine lysine arginine pattern of amino-aciduria only examples of this same type are encountered. Similarly, the generalized type of amino-aciduria runs true to type in individual families, and among the relatives of this group of patients the specific cystine lysine arginine pattern of amino-aciduria is no more frequent than in the general population.

Thus, both from the results of biochemical and familial investigations, there is every reason to believe that 'cystinuria' is not a homogeneous entity and cannot be regarded from either the biochemical or genetical standpoint as a unit character. It seems likely that the two groups, thus distinguished by the chromatography of the urinary amino-acids, will require further subdivision. Quantitative study, using the technique of polarography for the quantitative estimation of cystine in urine, has revealed familial differences in the group with the cystine lysine arginine pattern which suggest that more than one genetical type exists. Clinical and familial studies in the other group lead to similar conclusions. As yet no clear characterization of such subgroups has been achieved.

A third apparently quite distinct group of cases has also been identified in which there a generalized amino-aciduria sometimes involving and sometimes not involving cystine, but here the clinical features are those of the disorder first recognized by Wilson (1912) and known as hepato-lenticular degeneration. This is a hereditary disease in which pathological changes

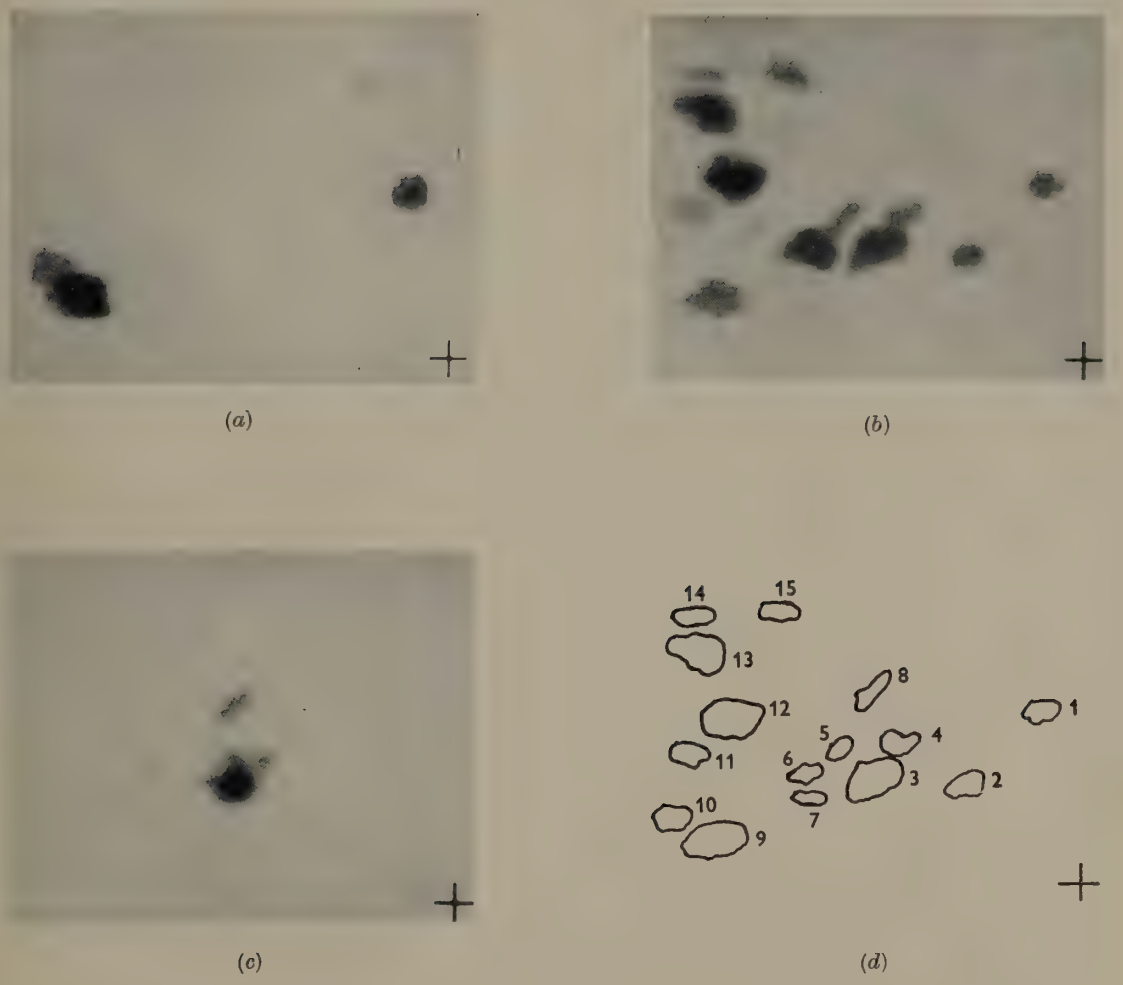


Fig. 3. Chromatograms of urine from: (*a*) Cystinuric individual showing the typical cystine, lysine, arginine pattern. (*b*) Patient with the Fanconi Syndrome showing generalized amino-aciduria. (*c*) normal individual. In each case a volume of urine containing 250 mg. of nitrogen was placed on the cross in the right-hand corner, and then phenol run from right to left as a first solvent, followed by collidine-lutidine in an upward direction. The spots show the amino-acids in their final positions after development with ninhydrin. (*d*) Key to the spots: (1) cystine as cysteic acid, (2) glutamic acid, (3) glycine, (4) serine, (5) threonine, (6) alanine, (7) glutamine, (8) taurine, (9) lysine, (10) arginine, (11) proline, (12) valine, (13) leucine or isoleucine or both, (14) phenylalanine, (15) tyrosine. (After Dent & Rose, 1951.)

are found characteristically in the basal cerebral nuclei and in the liver. It bears no relation to the Fanconi syndrome and presumably represents a distinct type of amino-aciduria which, according to whether cystine is more or less prominent among the amino-acids excreted, may or may not come under the classification of 'cystinuria' (Dent & Harris, 1951).

METHAEMOGLOBINAEMIA

A more subtle example of how quite diverse genetically determined chemical processes can give rise to similar end results is provided by the work that has been done on congenital 'idiopathic' methaemoglobinaemia.

Methaemoglobinaemia is a condition in which a substantial proportion of the intracellular haemoglobin exists as methaemoglobin, that is, pigment in which the haemoglobin iron is in the ferric state and is incapable of transporting oxygen. This condition may occur in normal people as a transient phenomenon due to the toxic effects of certain drugs such as acetanilide, antipyrine and phenacetin. The methaemoglobin disappears from the blood in 24–72 hours after stopping the drug. However, an entirely different form of the condition, known as congenital idiopathic methaemoglobinaemia, has been recognized for many years. It is generally present at birth and persists unchanged, in the absence of specific treatment, throughout life. It has frequently been found in several members of the same family and there is little doubt of its genetical determination (Hitzenberger, 1933; Bensley, Rea & Mills, 1938; Barcroft *et al.* (1945); Deeny, Mundrik & Rogan, 1943; Lian *et al.* 1939). The quantity of methaemoglobin present in these people generally amounts to several grammes per 100 ml. of blood and may represent from 10 to 45 % of the total red-cell pigment. Such individuals show a marked cyanosis, slaty blue in appearance. In most cases they appear to suffer from no other physical disability at all, and are able to lead perfectly healthy and active lives without any specific medical treatment. Other cases show some degree of dyspnoea on effort and there may be a compensatory polycythemia. Occasionally more severe disability such as dwarfing and mental defect may be associated with the disorder.

The condition is extremely rare. Probably not more than fifty examples have been described in the last twenty years in the medical literature, in spite of the fact that the individuals concerned have such a remarkable and striking appearance that the anomaly can readily be recognized. The evident rarity of this condition makes it perhaps even more remarkable that at least two quite distinct types have been characterized biochemically and genetically and even these may well not account for all the known cases.

A family extensively studied by Gibson & Harrison (1947) illustrates one of these types (Fig. 4). There were nine brothers and sisters, five of whom were definitely affected, the mother had died some years previously but was stated never to have been cyanosed. The father was perfectly normal. None of the affected brothers and sisters suffered from any physical disability from the presence of the abnormal pigment. The family lived in a remote and mountainous part of Ireland. The families of both parents had lived in the village for generations and were likely to have been in some degree consanguineous. No other relatives

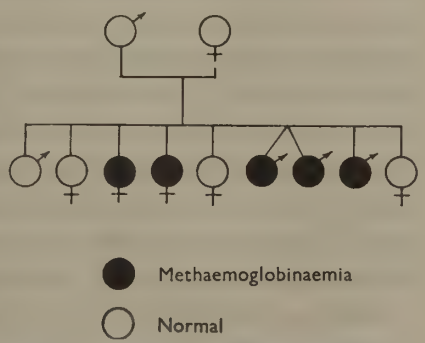


Fig. 4. Pedigree of methaemoglobinaemia. (After Gibson & Harrison, 1947.)

on either side of the family had had the cyanotic appearance. It seems probable, therefore, that the condition was being inherited as a rare recessive character.

Darling & Roughton (1942) have shown that in haemoglobin solutions containing methaemoglobin produced artificially there is a shift of the oxygen dissociation curve of the haemoglobin to the left. Precisely the same kind of shift was found to occur in the blood from these patients (Fig. 5). In other words, the haemoglobin gave up oxygen less readily than is normal at a given oxygen pressure. Darling & Roughton interpreted this shift as being due to the presence in haemoglobin-methaemoglobin mixtures of molecules containing both ferrous and ferric atoms in various atomic proportions. This shift has a bearing on the mode of production of the abnormal pigment since it implies that the methaemoglobin is distributed throughout the corpuscles rather than segregated in certain ones. If there were two kinds of corpuscle in the circulation, one containing normal 'ferrous' haemoglobin molecules and one containing only methaemoglobin, there would be no opportunity for the two types of pigment to interact and so the oxygen dissociation curve would be normal in position.

TABLE 9. *Findings in family (Fig. 4) with methaemoglobinaemia*

After Gibson & Harrison (1947).

No.	Age (years)	Colour of skin	Initial Met-Hb (g./100 ml.)	Total pigments (g./100 ml.)	Met-Hb as % of total pigments	Met-Hb after treatment (g./100 ml.)
Father	67	Normal	0·0	14·6	0	.
1	32	Normal	.	.	.	.
2	31	Normal	0·0	15·6	0	.
3	28	Lavender	3·1	15·3	20	0·1
4	27	Grey-blue	1·9	15·9	12	0·3
5*	.	.	.	.	.	.
6	22	Slaty blue	4·8	18·7	26	1·3
7	22	Slaty blue	5·1	18·8	27	0·5
8	21	Grey-blue	1·9	17·4	11	0·1
9	18	Normal	0·1	12·7	1	.

* Died of pertussis at 7 months.

In the normal erythrocyte it is thought that there is a steady formation of methaemoglobin, but the cells are capable of reducing it as rapidly as it is formed. An equilibrium Hb $\rightleftarrows$ Met Hb is set up and is generally well over to the left, only about 0·4 % of the pigment of the normal red cell being present as methaemoglobin (van Slyke, Hiller, Weisiger & Cruz, 1946). It seems that the abnormality in the red cells in these patients is concerned with a specific failure of the enzyme systems normally concerned with the reduction of the methaemoglobin which is constantly being formed. This leads to a displacement of the equilibrium Hb $\rightleftarrows$ Met Hb to the right and the consequent accumulation of abnormal amounts of methaemoglobin in the blood. The nature of this enzyme system and its abnormality in the affected members of this family has been extensively studied by Gibson (1948).

It appears that the reduction of methaemoglobin is normally coupled with glycolysis. In the glycolytic process coenzyme I (diphosphopyridine nucleotide) is reduced and is available for the reduction of methaemoglobin. Reduced coenzyme I will, however, alone only reduce

methaemoglobin at a very limited rate, and apparently in normal cells a flavoprotein (coenzyme factor I) acts as a carrier between coenzyme I and the methaemoglobin. Gibson found that in the red cells of these individuals with methaemoglobinaemia there was no defect in the part of the cycle concerned with the formation of the reduced coenzyme I. There was, however, a significant deficiency of coenzyme factor I activity, and he concluded that this was the primary defect in the red cells and led directly to the disturbance in the Hb ⇄ Met Hb equilibrium (Fig. 6).

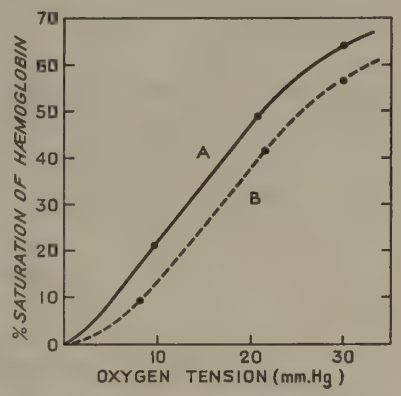


Fig. 5. Oxygen dissociation curve of blood in methaemoglobinaemia: A, with blood containing 4·2 g. of methaemoglobin per 100 ml. of blood, B, with blood after removal of methaemoglobin with methylene blue. (After Gibson & Harrison, 1947.)

The percentage of methaemoglobin could be dramatically reduced in these individuals by treatment with ascorbic acid or methylene blue by mouth. The action of the ascorbic acid is probably due to a direct reaction between methaemoglobin and ascorbic acid (Gibson, 1943). This reaction is comparatively slow and, even with the greatest concentration of ascorbic acid it is possible to obtain in blood, the displacement of the equilibrium Hb ⇄ Met Hb is not sufficiently great to lead to a complete disappearance of the methaemoglobin. Cases treated in this way tend to settle down at a level of abnormal pigment of about 1 % of the total. The action of the methylene blue is much more rapid and complete, and appears to depend on the catalytic effect causing methaemoglobin to be reduced by enzyme systems which are otherwise unable to bring about this reaction or alternatively do this at too slow a rate. In particular, Gibson (1948) suggests that it opens up pathways via coenzyme II and coenzyme factor II (Fig. 6).

What appears to be both genetically and chemically an entirely different variety of chronic methaemoglobinaemia occurred in a family described by Hörlein & Weber (1948). This condition resembles the type mentioned above in that some 15–25 % of the red-cell pigment was in the form of methaemoglobin and the individuals concerned seemed to be quite fit and showed no obvious ill effects from the condition. It differed, however, in several important respects. It occurred in four generations of the same family, apparently being transmitted from parent to child. This suggests that the affected individuals were heterozygous and not, as appeared likely in the family of Gibson & Harrison, homozygous. The methaemoglobin in these cases was found to have a different spectral absorption curve from normal methaemoglobin (Fig. 7). By splitting the haem and globin components and recombining them with the fractions from a normal person, it was possible to show that the abnormality lay in the globin moiety. Finally, it was found that treatment with ascorbic acid or methylene blue failed to bring about the striking therapeutic response encountered in the other type of case.

Thus it seems probable that the same end result, methaemoglobinaemia, may occur from two genetically quite distinct processes. In the first type there is some failure in the synthesis of a flavoprotein normally necessary for the maintenance of haemoglobin in the ferrous state in the erythrocyte. In the second type the peculiarity appears to reside in the synthesis of the globin, the different character of which leads presumably to the excessive formation of methaemoglobin in the erythrocytes.

There may be other types of methaemoglobinaemia. Eder, Finch & McKee (1949) have

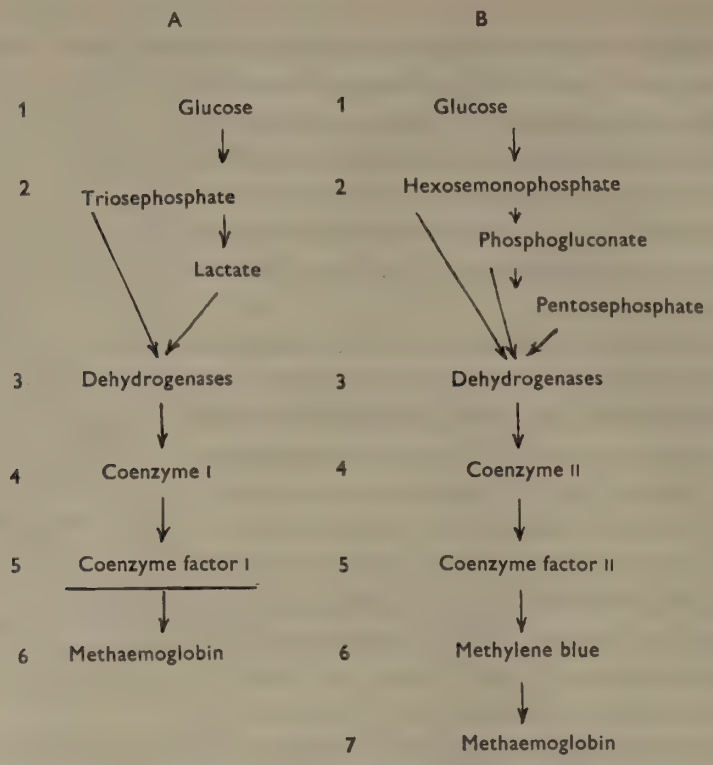


Fig. 6. Diagrammatic scheme of the reactions taking place in the reduction of MHb in erythrocytes. Column A reactions occurring in the absence of methylene blue; B in the presence of methylene blue. In cases of idiopathic methaemoglobinaemia where there is a deficiency of coenzyme factor I, the reactions in column A do not occur, and MHb reduction is very slow. (Gibson, 1948.)

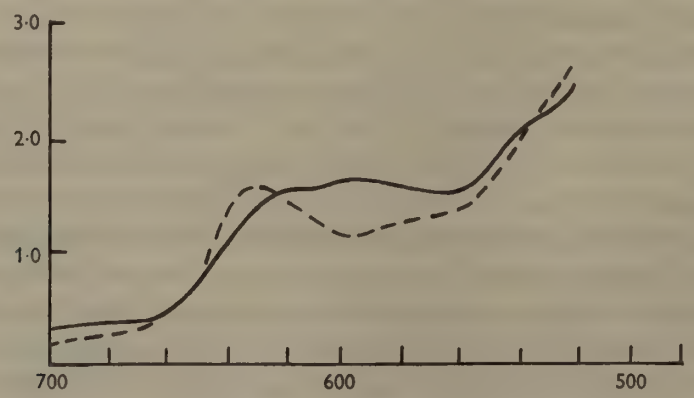


Fig. 7. Absorption curve of abnormal (continuous line) and normal (discontinuous line) methaemoglobins. (After Horlein & Weber, 1948.)

studied a patient with some 30–45% methaemoglobin. This patient differed from the types described by Gibson & Harrison (1947) in that the oxygen dissociation curve of his blood failed to show the shift described by Darling & Roughton (1942). On the other hand he responded well to treatment with ascorbic acid and methylene blue and so differed from the type of patient described by Hörlein & Weber (1948).

ALKAPTONURIA

Heterogeneity may sometimes be inferred from purely genetical considerations and in the absence of any direct biochemical evidence. The situation in alkaptonuria affords a typical example of the line of reasoning involved.

It has been pointed out that the familial distribution found in most cases of alkaptonuria is consistent with the hypothesis that they are determined by a single recessive gene, and the occurrence of a very high incidence of parental consanguinity makes such a conclusion almost certain. However, several pedigrees of the condition have been reported (Pieter, 1925;

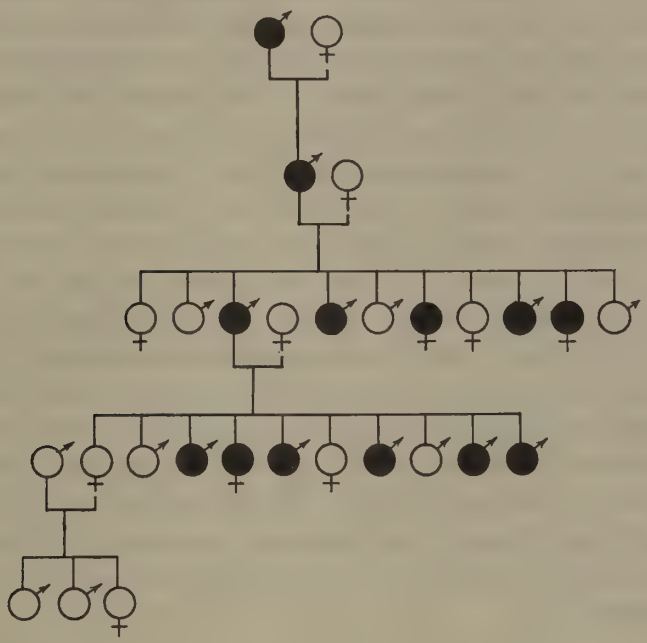


Fig. 8. Pedigree of heterozygous alkaptonuria. (After Pieter, 1925.)

Toenissen, 1922; Hogben *et al.* 1932) which do not fit this interpretation. In the pedigrees, individuals in a series of successive generations of a family were affected and there was no indication of any consanguineous marriages (Fig. 8). In other words the pedigrees were of typical heterozygous or 'dominant' type. The hypothesis that the affected members of these families were homozygotes like other alkaptonurics would imply a series of matings with heterozygous individuals which, in the absence of consanguineous marriage, would be extremely improbable. The simplest view is that there are two types of alkaptonuria—the usual type determined by a recessive gene, and a rare type, perhaps representing only a few per cent of all cases, in which the affected individuals are heterozygous for some other gene. Whether the gene involved in the second type of case is allelic with the first or is at some different chromosomal locus is not known. The existence of such genetical heterogeneity in alkaptonuria naturally raises the question of whether the two types are distinct biochemically. All that is known is that the rare type excretes homogentisic acid continuously and in large quantities. It is quite possible that the nature of the biochemical disturbance leading to this may be entirely different

from that found in most alkaptonurics. Since all the detailed metabolic studies so far conducted have naturally been done on the relatively common type of the disorder there is no definite information available as yet on this point.

THE BLOOD GROUPS A_1 AND A_2

Even in the blood groups, where the degree of specificity implied by serological characterization is considerably greater than in most other fields, it often happens that what was originally thought to be a single antigen common to a group of individuals is found, with the discovery of a new serum, to consist of more than one distinct type. Von Dungern & Hirzfeld (1911), for example, found that when anti-A serum from a group B blood was absorbed with certain group A bloods until it lost the power of agglutinating the absorbing red cells, the serum still agglutinated most other bloods of group A and group AB. The implication of this kind of experiment that the antigen A is not a single substance but includes two distinct compounds with different antigenic properties is now generally accepted, although certain authors have suggested that the difference may be a quantitative rather than a qualitative one. At any rate it is possible, on the basis of these results, to distinguish the two subgroups of A, A_1 and A_2, and similarly the two subgroups of AB, A_1B and A_2B. There are two antibodies: α which reacts with both A_1 and A_2 and α_1 which is specific for A_1 and does not react with A_2. The two subdivisions of A appear to be inherited as discrete units. Thus Wiener (1943), in his extensive survey of the published material, found that in thirty-eight matings of $A_1B \times O$ parents there were fifty-five children of group A_1 and fifty-three of group B, while in sixteen matings of $A_2B \times O$ parents there were thirty-seven A_2 children and twenty-seven of group B.

Thomsen, Friedrenreich & Worsaae (1930) suggested that Bernstein's original theory could be extended to account for these subgroups of A. They proposed the hypothesis of four allelic genes, $\mathbf{A_1}$, $\mathbf{A_2}$, **B** and **O**, and Wiener's (1943) collected data on over 1000 families substantially confirm this hypothesis. The combinations of the four alleles giving rise to the observed six phenotypes are as follows:

Genotype	Phenotype
$\mathbf{A_1A_1}$, $\mathbf{A_1A_2}$, $\mathbf{A_1O}$	A_1
$\mathbf{A_2A_2}$, $\mathbf{A_2O}$	A_2
BB, **BO**	B
$\mathbf{A_1B}$	A_1B
$\mathbf{A_2B}$	A_2B
OO	O

Using the sera generally available, $\mathbf{A_1}$ can be said to be dominant to $\mathbf{A_2}$ and **O**, $\mathbf{A_2}$ is dominant to **O**, and **B** is dominant to **O**.

CONCLUSION

The progressive differentiation of apparently discrete and homogeneous conditions or characters is likely to prove of increasing importance in this subject. Each problem has to be considered with this in mind and a combined biochemical and genetical approach will be found necessary to resolve the difficulties. At present the formal demonstration that more than one genetical process is involved depends only on the consideration of the familial and population distributions of the particular 'character' concerned. It may be anticipated that in future linkage studies employing common characters, such as the blood groups, as chromosomal 'markers' will aid in the resolution of these difficulties, particularly in helping to decide whether we are dealing with allelic genes or genes at different loci.

IV. BLOCKS IN INTERMEDIARY METABOLISM

Garrod (1923) put forward a simple hypothesis to account for the diverse biochemical manifestations which may be observed in these inherited metabolic variations. He suggested that in each condition the body was unable to perform some particular step in the normal course of metabolism. This was due to the congenital absence of the enzyme concerned in performing the step in question and as a result a block in metabolism occurred. The abnormal concentrations of particular metabolites in the body fluids and the clinical signs and symptoms with which they were associated could all, he believed, be ultimately traced back to the inability to carry out this single step in the metabolic chain.

There is little doubt that this conception of an inborn metabolic block is an accurate and useful way of describing many of the biochemical abnormalities of the type that we have been discussing. In some cases it has been possible by careful analysis to discover the particular metabolic step which is at fault. However, in most cases the nature of the specific abnormality in the enzyme system involved is not understood.

Phenylketonuria, alkaptonuria, and the much less frequent condition tyrosinosis, are three closely related disorders which have been regarded as examples of such metabolic blocks. It is probable that they represent failures to carry through three quite distinct steps in the metabolic chain by which the benzene ring of the aromatic amino-acids is normally broken down in the body. A consideration of some of the evidence on which this view is based will perhaps serve to illustrate the main features of this conception of inborn blocks in metabolism.

PHENYLKETONURIA

Phenylketonuria was first recognized by Fölling (1934 *a*, *b*), who demonstrated the presence of large amounts of phenylpyruvic acid in the urine of several mentally defective patients. The presence of phenylpyruvic acid in these quantities ($\frac{1}{2}$–1 g. a day) may be readily shown by the addition of a few drops of 5 % ferric chloride solution to the urine. A deep bluish green colour develops which slowly fades over the next few minutes.

All individuals who have so far been found to excrete phenylpyruvic acid continuously in their urine have shown a greater or less degree of intellectual impairment. Generally this has been severe, amounting to idiocy or imbecility, but occasionally higher grade feeble-minded individuals have been encountered. The addition of ferric chloride is nowadays a routine procedure in urine examinations for ordinary clinical purposes, and it is likely that if phenylketonuria in the absence of mental defect occurred it would have been discovered in this way.

Neurologically phenylketonurics show no paralysis and no increase in muscular tone, but a constant and peculiar feature is a marked accentuation of all the reflexes, both superficial and deep. There is also slight reduction in stature and in head measurements as compared with the normal averages of the same age and sex. The other peculiar feature of this condition is a slight dilution of the hair and skin pigmentation.

Phenylpyruvic acid is, however, not the only substance present in abnormal quantities in the urines of these patients. Greatly increased quantities of phenylalanine and phenyllactic acid (Fölling, Closs & Gammes, 1938; Dann, Marples & Levine, 1943) are also found. Phenyl-

acetyl glutamine has also been identified as being present (Woolf, 1951). This is presumably derived from the detoxication of phenylacetic acid with glutamine. The other amino-acids occur in the urine in normal quantities, and so far no other substances have been reported as being present in abnormal concentrations.

The blood plasma shows a very high concentration of phenylalanine. Jervis, Block, Bolling & Kanze (1940) found values ranging from 15 to 45 mg. % in the bloods of sixteen phenylketonuric patients, Borek, Brecher, Jervis & Waelsch (1950), using microbiological methods, found, in eighteen patients, values ranging from 19 to 33 mg. % as compared with a value in normal individuals of $0{\cdot}95 \pm 0{\cdot}06$ mg. %. Although there was some variation in the values taken at different times from the same individual, it appeared that the individual variations were significantly smaller than the variations between individuals. This suggests that there may be a characteristic level of blood phenylalanine for each individual. The high phenylalanine concentration has also been demonstrated by chromatography on filter-paper by Dent (1950) who was able to show by this method that the levels of the other amino-acids in the blood were of the order of magnitude found in normal people.

Phenylalanine is also present in considerably increased concentrations in the cerebrospinal fluid of these patients (Jervis *et al.* 1940; Borek *et al.* 1950). The concentration here is of the order of 7 mg. % and this represents an increase over the normal values for cerebrospinal fluid of the same order of magnitude as that observed in the blood.

At the most only traces of phenyllactic acid, phenylpyruvic acid or phenylacetic acid occur in the blood or cerebrospinal fluid.

No peculiarity in the tissue proteins has been identified. Block, Jervis, Bolling & Webb (1940) prepared proteins from sera, erythrocytes, brains, liver and kidneys of normal individuals and from phenylketonurics, and analysed them for nitrogen, sulphur, histidine, arginine, lysine, cystine, tyrosine, tryptophan, threonine and phenylalanine. No significant differences were detected.

Thus, biochemically, the outstanding feature of phenylketonuria is the high level of phenylalanine in the blood, cerebrospinal fluid, and urine of affected patients. With this is associated the occurrence of phenylpyruvic acid, phenyllactic acid and phenylacetyl-glutamine in the urine (Fig. 9).

It is evident that this disorder represents a severe disturbance of phenylalanine metabolism, and this has been confirmed by the various metabolic experiments which have been conducted on affected individuals. When the patients were fed phenylalanine, a considerable increase in the excretion of phenylpyruvic acid was detected in the urine (Fölling *et al.* 1938; Penrose & Quastel, 1937; Jervis, 1938). A rise in the level of phenylalanine in the blood and cerebrospinal fluid was also observed (Jervis *et al.* 1940). Feeding other amino-acids, such as tyrosine, tryptophan, serine, dihydroxyphenylalanine, alanine, leucine, cystine and glycine produced no such effect (Jervis, 1938; Penrose & Quastel, 1937). When phenylpyruvic or phenyllactic acids were given to the patients there occurred an increased excretion of these acids in the urine (Penrose & Quastel, 1937; Jervis, 1938) and a rise in the level of phenylalanine in the blood (Jervis *et al.* 1940). There was, however, no phenylpyruvic or phenyllactic acid detected in the blood (Jervis *et al.* 1940). Phenylpropionic, phenylglyceric, cinnammic, *p*-hydroxyphenylpyruvic and homogentisic acids all failed, after feeding, to augment the excretion of phenylpyruvic acid in the urine (Jervis, 1938).

It seems, therefore, that the metabolic disorder is concerned primarily with the metabolism of phenylalanine, and that in these patients, as in normal individuals, phenylpyruvic acid, phenyllactic acid and phenylalanine are interconvertible.

Phenylalanine is an essential amino-acid. Rose, Haines, Johnson & Warner (1943) have shown that in man it is an indispensable dietary constituent, and that its omission from the diet is followed by a pronounced negative nitrogen balance. The body has no capacity to synthesize the benzene ring and this is mainly supplied in the diet by the aromatic amino-acids,

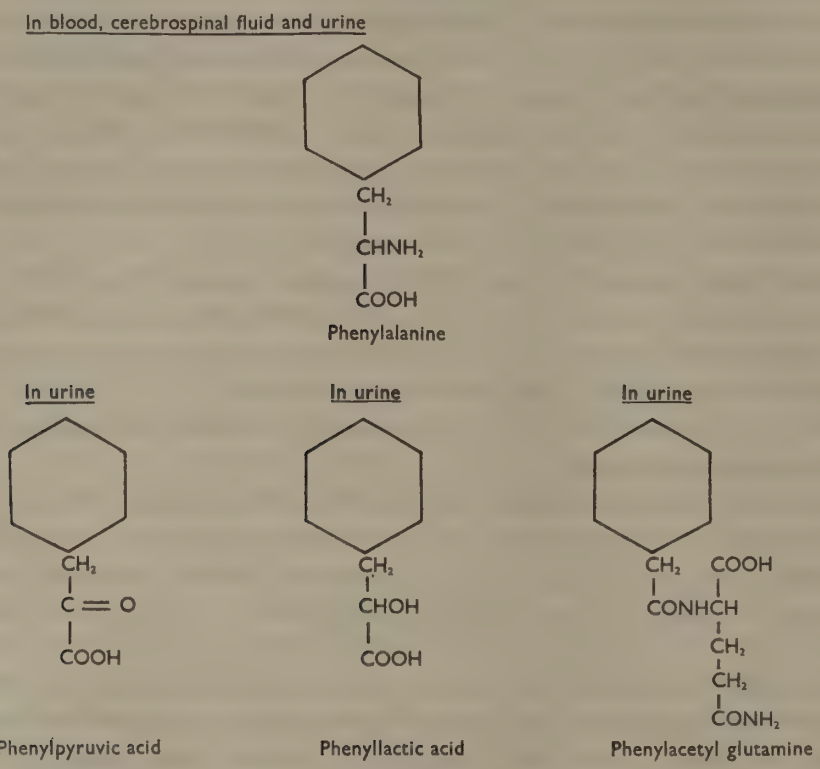


Fig. 9. Substances found in the body fluids in abnormal amounts in phenylketonuria.

phenylalanine and tyrosine. Tyrosine, however, is not essential (Rose *et al.* 1943), though it may become a limiting factor for growth and nutrition if the diet does not contain enough phenylalanine. Furthermore, tyrosine can be formed from phenylalanine (Fig. 10) and there is much evidence which suggests that this is an important pathway in the normal metabolism of phenylalanine. Moss & Schoenheimer (1940), for example, prepared DL-deutero-phenylalanine in which most of the deuterium was present in the benzene ring. The amino-acid was added to a casein stock diet and fed to growing and adult rats. Subsequently samples of tyrosine were isolated from the proteins of these animals and their deuterium content determined. The tyrosine isolated from the internal organs contained a concentration of deuterium indicating that about 20–30 % of this tyrosine was derived from the deutero-phenylalanine. In another experiment, non-isotopic tyrosine, in addition to the deutero-phenylalanine, was added to the stock diet of adult rats. Despite the abundance of tyrosine in the diet, about 13 % of the tyrosine in the internal organs was found to be derived from the deutero-phenylalanine. These

experiments showed that phenylalanine is rapidly converted into tyrosine, not only by growing, but also by fully grown rats of constant body weight.

The reaction appears to be irreversible. Womack & Rose (1934) found that tyrosine was entirely unable to replace phenylalanine as an essential dietary constituent, and Hier (1947) who studied the plasma levels of various amino-acids after feeding given individual amino-acids to dogs showed that there was a considerable rise in tyrosine level after L-phenylalanine, but the reverse did not occur. Further evidence of this sort is provided by the experiments of Levine, Dann & Marples (1943) who studied a series of healthy infants on a constant high protein vitamin C free diet. Various amounts of extra L-tyrosine and DL-phenylalanine were administered. Repeated ingestion of phenylalanine gave rise in the urine to phenylalanine, phenylpyruvic acid, tyrosine, *p*-hydroxyphenylpyruvic acid and phenyllactic acid representing 44–73 % of the extra phenylalanine added. On the other hand, feeding tyrosine gave rise to no phenylalanine or phenylpyruvic acid, although tyrosine and its keto and hydroxyacids were present in abundant amounts (40–80 % of the ingested dose).

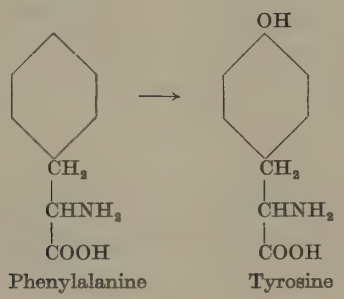


Fig. 10

Udenfriend & Cooper (1952) have demonstrated the presence of an enzyme system in mammalian liver capable of catalysing this conversion of phenylalanine to tyrosine. The system required both oxygen and pyridine nucleotide and was evidently not a simple one. At least two enzymes are thought to be involved.

It seems probable that the specific abnormality present in phenylketonuria is an inability to perform this particular step from phenylalanine to tyrosine (Fig. 10). Jervis (1947) demonstrated that in normal individuals feeding of phenylalanine or phenylpyruvic acid led to a rise in Millon-reacting substance (presumably tyrosine) in the blood. In phenylketonurics this did not occur (Fig. 11). After feeding tyrosine, however, a rise occurred both in phenylketonurics and in the normals. The processes of amination and deamination do not appear to be disturbed. This is indicated by the rise in blood phenylalanine after feeding phenylpyruvic acid and by the ready production of phenylpyruvic acid in the urine after feeding phenylalanine.

Furthermore, it is likely that the metabolic block is virtually complete. Dann *el al.* (1943), for example, found that after feeding extra phenylalanine to their patient, 98 % of the extra amount ingested could be accounted for within 48 hours by the increased excretion of phenylpyruvic acid, phenyllactic acid and phenylalanine.

Fölling *et al.* (1945), basing their ideas on the greater ease with which the organism forms phenylpyruvic acid from the D- rather than the L-phenylalanine, suggested that the primary step in the metabolic disorder was a transformation of L- to D-phenylalanine, and the subsequent

conversion of this to phenylpyruvic acid. However, they were unable to demonstrate the presence of D-phenylalanine in the blood or urine of their patients. Similarly, Prescott, Borek, Brecher & Waelsch (1949), using much more sensitive microbiological methods, were unable to demonstrate any D-phenylalanine in the plasma of their patients.

The cause of the mental defect is not understood. Himwich & Fazekas (1940) found that there is a diminished uptake of oxygen and glucose from the blood passing through the brain and that this is due to a decreased cerebral metabolism. It is not known how this is brought about,

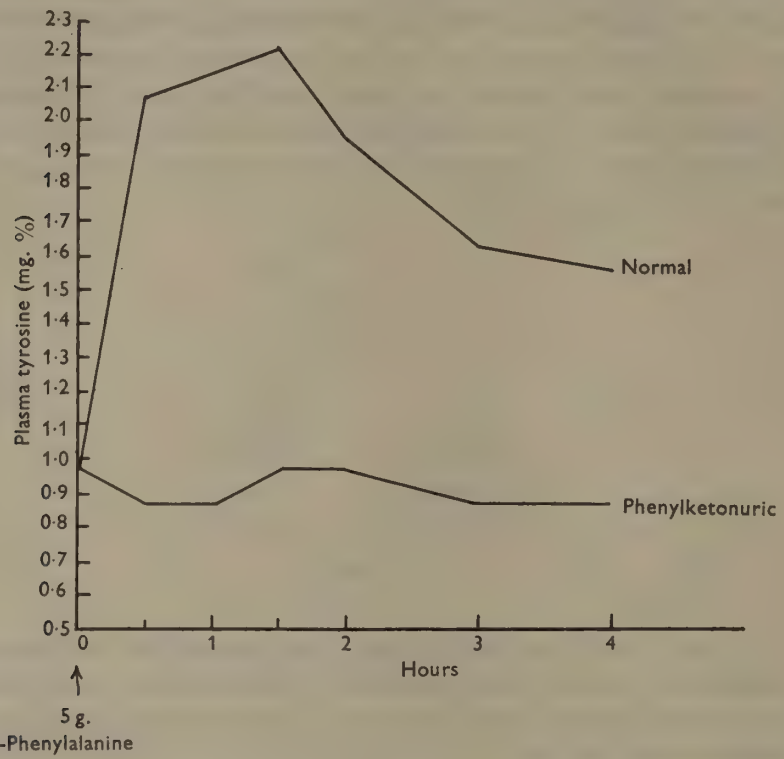


Fig. 11. Effect on plasma tyrosine of feeding 5 g. of phenylalanine to a normal and a phenylketonuric individual. (After Jervis, 1947.)

although the high levels of phenylalanine in the blood and cerebrospinal fluid suggest that this substance may in some way be exerting a toxic effect. However, although there have been occasional reports of particularly low concentrations of one or other phenylalanine derivatives being found in patients with relatively high intelligence quotients, there appears to be no overall correlation between the phenylalanine level in the blood, or its derivatives in the urine, and the mental performance as measured by I.Q. score.

We have no satisfactory way of accounting for the slight pigmentary disturbance which occurs, other than the general connexion between the aromatic amino-acids and melanin formation.

On the present evidence, the simplest explanation of the metabolic disturbance in phenylketonuria would appear to be the following. There is a specific inability in the organism to hydroxylate phenylalanine in the *para* position, so as to give rise to tyrosine. This is normally the first step in the oxidation of phenylalanine, and in its absence phenylalanine accumulates

in the body fluids and is excreted in the urine. The process of oxidative deamination is not disturbed and the increased concentration of phenylalanine consequently gives rise to the production of large quantities of phenylpyruvic, phenyllactic and phenylacetic acids, which are excreted in the urine, the last named combined with glutamine as phenylacetylglutamine. On this view, it would follow that tyrosine must be an indispensable dietary constituent for these patients, and in its absence, despite the presence of adequate phenylalanine in the diet, they should go into negative nitrogen balance. This possibility has, however, never been tested.

ALKAPTONURIA

The characteristic feature of alkaptonuria is the continuous daily excretion of several grammes of homogentisic acid in the urine. This substance was first isolated by Wolkow & Baumann in 1891. The actual quantity of this acid which is excreted varies with the diet, and is larger when the protein intake is increased. The excretion of homogentisic acid in these patients is highly correlated with the total nitrogen output, and the ratio homogentisic acid : total nitrogen excretion has been used as a convenient index in the study of these cases. Given a constant diet, this ratio is probably, on the average, much the same in all alkaptonurics (Garrod, 1923). Though, even on a standard diet, an individual case may show a certain amount of day to day variation (Table 10).

TABLE 10. *Daily excretion of homogentisic acid and nitrogen in an alkaptonuric on a basal diet*

After Neuberger *et al.* (1947).

Date	Vol. of urine (ml.)	Homogentisic acid output (g.)	Total nitrogen (g.)	H : N ratio
26–27 Jan.	640	4·87	9·1	0·535
27–28 Jan.	885	4·39	8·9	0·495
28–29 Jan.	715	4·65	9·75	0·477
30–31 Jan.	1135	5·14	9·94	0·517
31 Jan.–1 Feb.	1175	5·09	9·26	0·55
1–2 Feb.	1015	3·93	8·74	0·45
4–5 Feb.	1040	5·01	9·98	0·50
5–6 Feb.	975	4·19	8·73	0·48
6–7 Feb.	750	3·57	7·02	0·50
10–11 Feb.	780	5·12	9·99	0·512
11–12 Feb.	1290	5·23	11·19	0·468

Apart from homogentisic acid, no other substance has been reported as occurring in abnormal quantities in the urine of these patients. In particular, Neuberger, Rimington & Wilson (1947), in a search for other aromatic compounds, failed to find any abnormal quantities of tyrosine, *p*-hydroxyphenyllactic acid, *p*-hydroxyphenylpyruvic acid or phenylpyruvic acid. The reducing properties of the urine could be attributed entirely to the homogentisic acid present.

The exact concentration of homogentisic acid in the blood plasma of alkaptonurics is still somewhat uncertain though it is probably surprisingly low. Katsch & Metz (1927), Lanyar & Lieb (1931) and Neuberger *et al.* (1947) all obtained values of the order of 3 mg./100 ml. Such concentrations are so low as to be near the lower limits of the applicability of the methods used

and, moreover, minute amounts of reducing substances reacting like homogentisic acids are present even in the plasma extracts obtained from non-alkaptonuric individuals, so that the plasma homogentisic acid levels reported cannot therefore be considered as very accurate (Neuberger *et al.* 1947). On the other hand, Abderhalden & Falta (1903) claimed actually to have isolated the acid in the form of its lead salt from 300 ml. of blood of an alkaptonuric, but, as Neuberger and his colleagues point out, this finding must remain doubtful because neither the yield, melting-point, nor analysis of the lead salt are stated. No other abnormal constituents have been reported in the blood of these patients.

Homogentisic acid is believed to be an intermediate in normal metabolism. Normal individuals after taking by mouth amounts of the substance of the order of 5 g. appear to metabolize it completely and no homogentisic acid appears in the urine (Falta, 1904; Leaf & Neuberger, 1948). Embden (1893) did, in fact, succeed in producing a transitory alkaptonuria in himself by swallowing as much as 8 g. of the acid, but he found that smaller doses had no such effect.

In sharp contrast with the findings in the normal, homogentisic acid, when fed to alkaptonurics, is excreted in the urine almost quantitatively, in addition to that normally being put out by these subjects (Embden, 1893). It appears that the alkaptonuric is completely unable to metabolize this substance.

The simplest interpretation of the metabolic disorder in alkaptonuria is that the further oxidation of homogentisic acid, a normal intermediate in the breakdown of phenylalanine and tyrosine, is completely blocked, and that consequently homogentisic acid accumulates and is excreted in large amounts in the urine. Apparently the failure lies essentially in the body's inability to break down the benzene ring with hydroxyl groups in the 2: 5 positions.

This is the main, though not the only, metabolic pathway of the two aromatic amino-acids, phenylalanine and tyrosine. Some 80–90 % of these amino-acids present in the diet are converted by the alkaptonuric to homogentisic acid. A similar conversion rate is obtained when either extra L-phenylalanine or L-tyrosine are fed. Other pathways not leading through homogentisic acid do exist though they are probably quantitatively limited. Thus both adrenaline and 3:4-dihydroxyphenylalanine as well as diiodotyrosine and thyroxin are derived from tyrosine in the body.

Taking the view that homogentisic acid is a normal intermediary metabolite, the results of administering various aromatic acids to alkaptonurics may be expected to throw light on the intermediate metabolic steps which precede the formation of this compound. A substance which represents a link in the chain should be destroyed in the normal organism and should increase the output of homogentisic acid in the alkaptonuric. With this in mind, many different substances have been fed to alkaptonurics and the earlier literature is fully reviewed by Garrod (1923) and Neubauer (1928).

Both phenylalanine and tyrosine when fed to these subjects greatly increase the excretion of homogentisic acid in the urine, and there seems no doubt that these are the parent substances from which it is derived. Phenylpyruvic, phenyllactic and *p*-hydroxyphenylpyruvic acids likewise give rise to an increased elimination of homogentisic acid in alkaptonuric patients (Neubauer & Falta, 1904; Grutterink & van der Bergh, 1907). On the other hand, *o*-tyrosine and *m*-tyrosine do not increase the excretion of homogentisic acid (Blum, 1908) and neither do the *o*- nor *m*-hydroxyphenylpyruvic acids. Thus it seems that the presence of the hydroxyl group in the *para* position, as in tyrosine, is not only no hindrance to the change to homogentisic

acid but is probably essential, and the transformation occurs not by removal of the hydroxyl group in the *para* position but by a shifting of the side chain.

Several different metabolic routes appear possible (Neubauer, 1928; Neuberger *et al.* 1947) (Fig. 12).

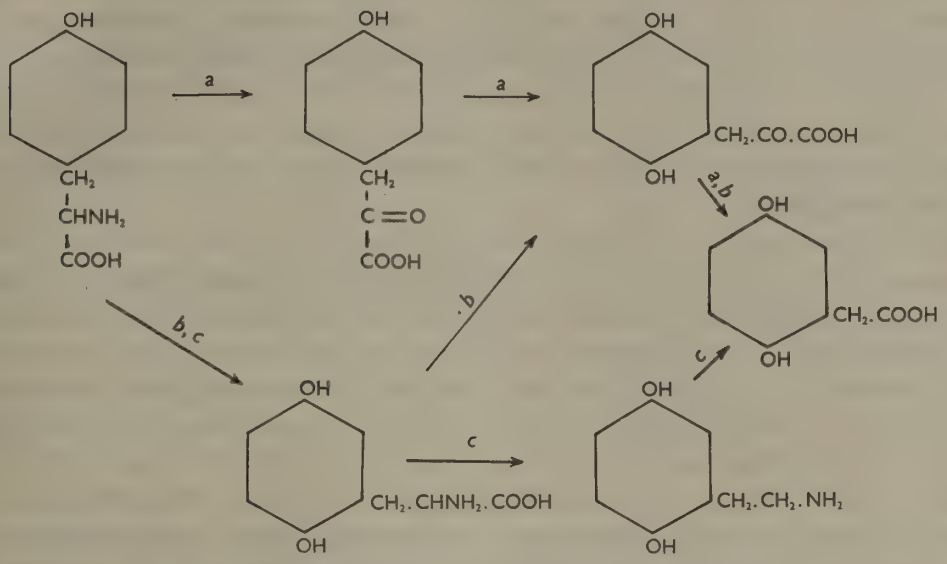


Fig. 12. Possible pathways in the metabolism of tyrosine.

It has generally been assumed that tyrosine is first converted to the corresponding keto-acid. This is further oxidized to 2:5-dihydroxyphenylpyruvic acid, which on oxidative decarboxylation would give homogentisic acid (*a*). However, *p*-hydroxyphenylpyruvic acid is far less effective than tyrosine in producing homogentisic acid in the alkaptonuric (Neubauer, 1909; Fromherz & Hermanns, 1914) and the amount of extra homogentisic acid excreted could be ascribed to its reconversion first into tyrosine by amination. Neuberger *et al.* (1947), therefore, as a result of their experiments on feeding 2:5-dihydroxyphenylalanine, in which a considerable increase in homogentisic acid excretion was produced in an alkaptonuric, suggest that the pathway *b, c* is probably the most important.

One curious feature of the biochemistry of this disorder is the high concentration of homogentisic acid found in the urine, when there is apparently a very low concentration in the plasma. It is probable that even in normal individuals there is a low renal threshold for homogentisic acid. Leaf & Neuberger (1948) were able, by intravenous injection of quantities of the order of 0·3 g. of homogentisic acid, to produce a transient alkaptonuria in normal individuals. On the average, about one-third of the dose administered appeared in the urine. The concentration in the plasma was of the order of 1·5–3·5 mg. %, and they concluded that the renal threshold for this substance must be well below 4 mg. %. In normal subjects, alkaptonuria cannot be produced by feeding tyrosine or phenylalanine, and it must be assumed that homogentisic acid, if it is formed as an intermediate, never appears in the blood in appreciable quantities, and it may well be rapidly oxidized in the organ where it first formed. The alkaptonuric probably has the same low renal threshold for this substance as does the normal and this could account for a high urine concentration and a low concentration in the plasma. However, the values for the renal clearance actually obtained by Neuberger *et al.* (1947) in the

patient they studied were remarkably high, about 400–500 ml./min. This is much more than could be readily accounted for by filtration alone, as a substance which was filtered by the glomeruli and not at all reabsorbed by the tubules might have been expected to show a clearance volume of 100 ml./min. at the most in this patient. Two possible explanations for these findings are offered. One is that the homogentisic acid is actually made in the kidney and passes immediately into the urine; the other that the homogentisic acid is made in some other organ, appears in traces in the blood, and is actively secreted by the kidney tubules at a rate comparable to that shown by certain dyes such as diodrast. Further work on the site of the formation and the mechanism of excretion of this substance needs to be done before this problem can be adequately resolved.

Apart from the excretion of homogentisic acid in the urine, the most notable clinical feature of alkaptonuria is the development of the condition known as ochronosis. This does not generally become apparent till middle or late life, and is due to the development of a dark pigmentation of the cartilages, tendons and ligaments, and of the sclerotics of the eyes. The earliest signs are a bluish discoloration of the ears, and the appearance of triangular brown patches in the sclerotics with their bases towards the corneae. Later, owing to staining of the nasal cartilages, the nose may appear bluish and a blue tint may develop on the knuckles due to staining of the underlying tendons. The post-mortem appearances are very striking. The cartilages and fibrocartilages are deeply pigmented, the staining of the tracheal rings and of the interstitial disks being particularly noticeable. Pigmentation of the tendons, sclerotics, and, in advanced cases, the bones may be observed, and patches of pigment in the endocardium and the intima of the arteries have also been described. The origin of the pigment is assumed to be homogentisic acid, but its actual mode of formation is not known in any detail.

The above changes do not appear to lead to any particular disability. However, it has also been observed that alkaptonurics are more prone than the average to develop arthritic changes in later life. These changes are most prominent in the spine, and they may lead to rigidity and kyphosis. Typical X-ray appearances have been described by Pomeranz, Friedmann & Tunick (1941), the main features of which are an extensive calcification in tendon sheaths, bursal sacs and synovial membranes. The arthritic changes are not necessarily related to the pigmentary changes, as it has been observed that the cartilages of affected joints may show little or no staining, and intense blackening of articular cartilages is consistent with a complete absence of arthritic lesions (Garrod, 1923).

TYROSINOSIS

Only one authenticated case of tyrosinosis has been described (Medes, 1932). It was, however, studied in considerable detail, and it clearly falls into place as a condition closely resembling phenylketonuria and alkaptonuria. In this patient there was a continuous excretion in the urine of large quantities of *p*-hydroxyphenylpyruvic acid and tyrosine. If the protein intake was increased or if tyrosine itself was fed, the amount of these compounds excreted was increased and also there appeared a certain amount of *p*-hydroxyphenyllactic acid. When the tyrosine intake was raised further, 3:4-dihydroxyphenylalanine could be demonstrated in the urine.

If phenylalanine was fed the excretion of tyrosine and *p*-hydroxyphenylpyruvic acid was increased. *p*-hydroxyphenylpyruvic acid on feeding mainly reappeared unchanged, but was also partly excreted as *p*-hydroxyphenyllactic acid. The intake of 3:4-dihydroxyphenylalanine led to an increase in the excretion of tyrosine and *p*-hydroxyphenylpyruvic acid. In contrast to

these findings, it was observed that on feeding homogentisic acid, this substance did not appear in the urine and there was no indication that it influenced the excretion of any other compound. Presumably it was completely metabolized.

These observations are consistent with a condition in which there is an inability to convert tyrosine to 2:5-dihydroxyphenylalanine or *p*-hydroxyphenylpyruvic acid to 2:5-dihydroxyphenylpyruvic acid. In other words, the block lies between those located for phenylketonuria and alkaptonuria.

The patient concerned also suffered from myasthenia gravis. However, many other cases of myasthenia gravis since investigated have failed to show the same biochemical peculiarities. It is perhaps worthy of note that the patient showed no obvious abnormality of pigmentation.

The three conditions phenylketonuria, tyrosinosis and alkaptonuria can be regarded as arising from blocks in successive stages of the series of reactions, which in the normal course of events leads to the breakdown of the benzene ring of the amino-acids, phenylalanine and tyrosine, in the body. A schematic representation of this process is shown in Fig. 13 and the suggested site of the three blocks is indicated. In none of these disturbances have we any knowledge of the precise manner in which the enzyme system concerned is at fault.

OTHER CONDITIONS

Many other conditions are known in which the metabolic disorder is probably attributable in the same way to a block in the normal metabolic pathways. Among these are fructosuria, L-xyloketosuria, congenital porphyria, glycogen disease, albinism, and the lipoidoses such as Niemann Pick's disease, Gaucher's disease, and amaurotic family idiocy (Tay-Sachs disease). In general, none of these conditions has been examined in such detail as those described above. However, metabolic studies in certain of these disorders have drawn attention to some interesting points.

FRUCTOSURIA

Fructosuria probably represents a condition in which only one of several possible pathways in the metabolism of fructose is blocked. As a result the organism is able to metabolize satisfactorily most of the fructose with which it has to deal and only a certain proportion of this substance fails to be utilized.

In this condition, some 10–20 % of the fructose taken in the food is excreted in the urine. The proportion appears to be relatively constant and independent of the total amount of fructose taken. This was first noted by Schlesinger (1903), and has been found in all subsequent studies where the appropriate investigations were made (Table 11). If the individual is on a diet free from fructose, or if he is fasting, no sugar appears in the urine, and no sugar appears after the ingestion of glucose or glucose-forming polysaccharides. The results of fructose tolerance tests indicate that there is some failure in the intermediary metabolism in such individuals and the fructosuria is not a renal threshold effect. After 50 g. of fructose, the blood fructose rises to much higher levels in fructosuria than in normal individuals and it disappears from the body more slowly (Fig. 14).

It is known that in normal subjects the ingestion of fructose leads to a rise in respiratory quotient which occurs more quickly and reaches a higher level than when glucose is ingested (Deuel, 1936; Bachmann & Haldi, 1937). This rise in respiratory quotient is associated with a marked elevation in the level of blood lactic acid, which occurs after the ingestion of fructose

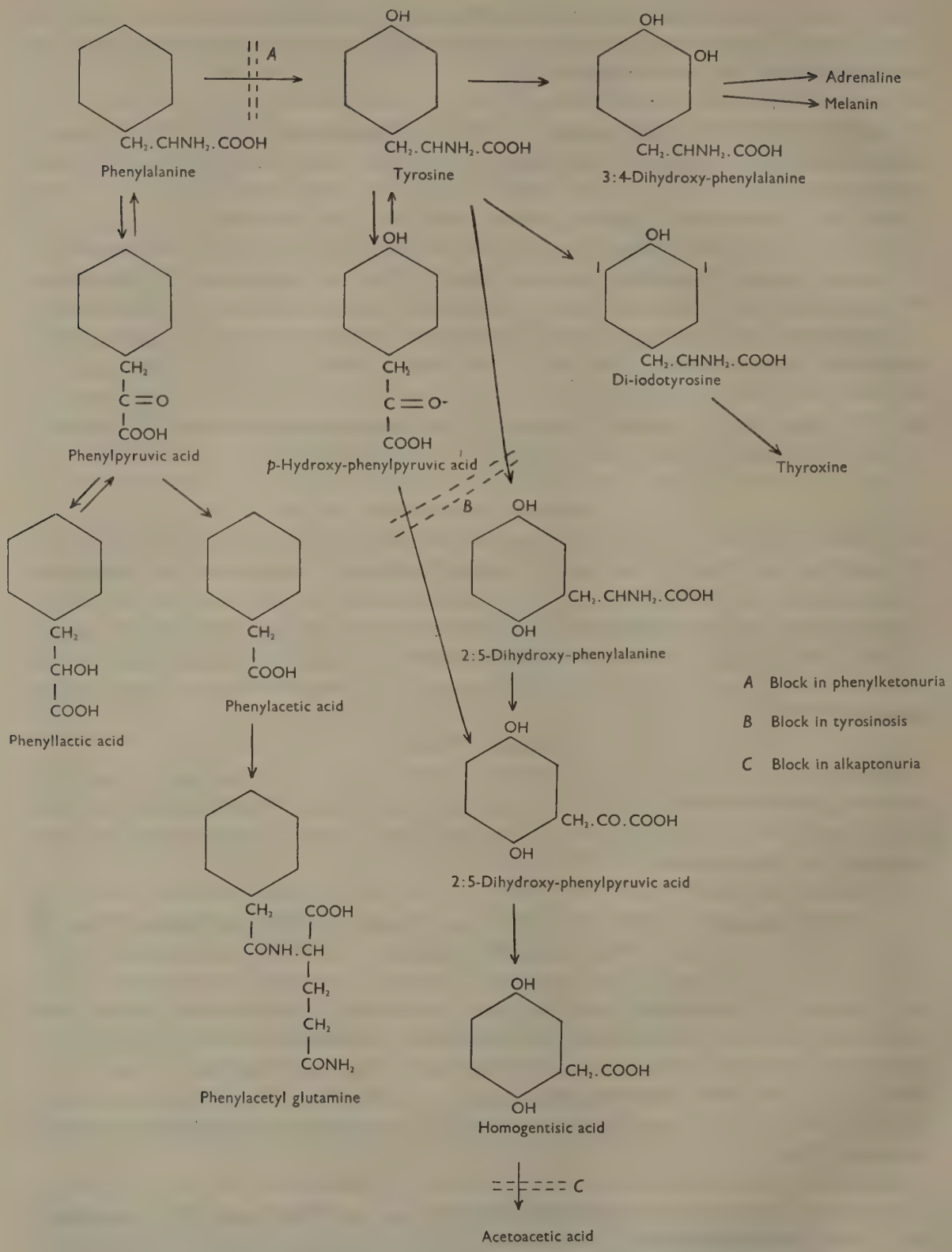


Fig. 13. Site of metabolic blocks in phenylketonuria, alkaptonuria, and tyrosinosis.

but not of glucose. Both these phenomena have been studied in fructosurics. It appears that here the respiratory quotient was only slightly raised, in a manner similar to that observed after glucose had been fed to normal subjects (Heeres & Vos, 1929; Soisalo, 1933; Rynberger, Chambers & Blatherwick, 1941). Similarly, little or no increase is observed in the level of blood lactic acid (Edham, Erden & Steinitz, 1938; Sachs, Sternfeld & Kraus, 1942; Rynbergen *et al.* 1941) (Fig. 14).

TABLE 11. *Amount of fructose excreted by a fructosuric after ingesting different amounts of fructose*

After Silver & Reiner (1934).

Urinary excretion of ingested fructose	
Fructose ingested (g.)	% of fructose excreted in 24 hr.
1	Traces
5	16
25	11
50	12
75	14
100	14

Sachs *et al.* (1942) explain these findings in the following way. They postulate that in normal individuals about 80 % of the ingested fructose is converted to glycogen, the remainder being broken down to lactic acid. Fructosurics are unable to deal with the part of the ingested fructose which normally goes to lactic acid, and so 10–20 % of the ingested fructose is excreted in the urine. However, further work appears to be necessary before this view can be substantiated.

GLYCOGEN DISEASE

In certain conditions where a block in metabolism is present, the metabolite immediately preceding the metabolic step which is at fault may accumulate in large quantities in particular tissues and cannot be excreted. This appears to be the situation in glycogen disease, and in certain of the lipoidoses.

In glycogen disease, first described by von Gierke (1929), there occurs an abnormal deposition of glycogen in the liver and sometimes also in the kidneys and the heart. The outstanding feature is a gross enlargement of the liver, and it is this which generally first directs attention to the disorder. Generally, growth tends to be delayed and, as a result, the affected children, while being of normal proportions at birth, lag behind children of the same age. If they survive into adult life the retardation in growth may be much less evident (van Creveld, 1952). The condition appears to be inherited as a simple recessive character.

There are four characteristic biochemical findings. The fasting blood sugar is consistently low, usually about 60 mg./100 ml. It fails to rise appreciably after the injection of adrenaline. There tends to be a rapid development of hypoglycaemia and ketosis on fasting. The liver contains grossly excessive quantities of glycogen (12–15 % of the wet weight) (van Creveld, 1939;

Bridge & Holt, 1945). The liver glycogen is abnormally stable after death and when biopsy material is allowed to stand *in vitro* for many hours little or no breakdown of glycogen occurs. When mixed with normal liver material, however, the glycogen breaks down rapidly. These

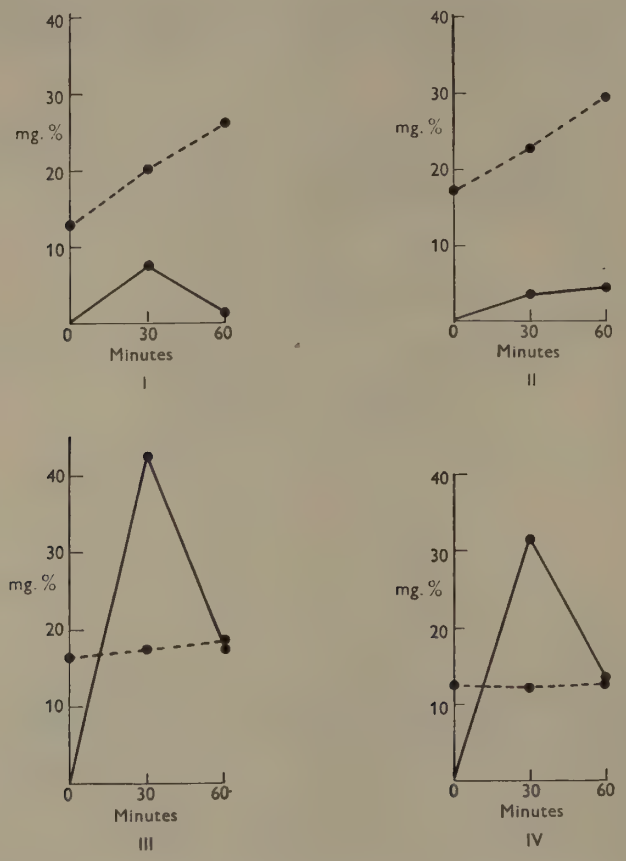


Fig. 14. Effect of feeding 50 g. of fructose to two normal controls I and II, and two fructosurics III and IV on blood fructose and lactic acid. ——, fructose; - - - - -, lactic acid. (After Sachs, Sternfeld & Kraus, 1942.)

findings indicate that there is an inability to break down glycogen once it has been stored in the liver and other tissues. This is presumably due to the absence of some enzyme usually present (Ellis & Payne, 1936). The enzyme concerned may be a specific phosphatase of glucose-6-phosphate (Cori & Cori, 1952).

V. VARIATIONS IN RENAL TUBULAR FUNCTION

The occurrence of an inherited disorder characterized by the excretion of abnormally large quantities of some particular metabolite in the urine cannot always be interpreted in terms of the conception of a block in intermediary metabolism. Such a situation may also arise as a result of some specific peculiarity in kidney function. That this is the explanation of the condition called renal glycosuria has been known for many years, and it seems probable that this particular peculiarity is by no means unique and that many other similar types of genetically determined variants exist.

RENAL GLYCOSURIA

Glucose is present only in traces in normal human urine. In certain diseases, the most prominent of which is diabetes mellitus, where there is a gross disorder of carbohydrate metabolism, the level of glucose in the blood may become greatly increased and consequently large quantities appear in the urine. Some individuals, however, who in other respects appear to be quite healthy, and who show no obvious abnormality of carbohydrate metabolism or undue elevation of blood-sugar levels, excrete glucose in their urine in quite large amounts. Such individuals are said to be suffering from renal glycosuria and it is believed that the essential abnormality is a specific impairment of the capacity of the renal tubules to absorb glucose from the glomerular filtrate. In this condition the blood sugar, after the administration of 50 g. of glucose by mouth, rises no higher than in the normal, and falls again to the fasting level within the normal time. This normal glucose tolerance test indicates that the utilization of glucose by the tissues is unimpaired. Typical results obtained with this test in normal and glycosuric members of the same family are shown in Table 12. Glycosuria, which in normal individuals will only occur if the blood sugar is elevated to values of the order of 160–180 mg. %, appears in these individuals at much lower levels. In certain cases the glycosuria occurs even at fasting blood-sugar levels, in others only if the blood sugar is slightly elevated above this. Consequently, gradations of the condition occur between those where the glycosuria is a constant feature and is present even when the individual is fasting, and those where it is intermittent and is only found after a carbohydrate meal.

In the mammalian kidney glucose passes freely into the glomerular filtrate and is normally practically completely reabsorbed by the renal tubules. The reabsorption is not due simply to diffusion, but is an active process possibly involving phosphorylation. It can be temporarily blocked by the administration of phloridzin. Shannon (1939) has postulated that during tubular reabsorption glucose enters into a reversible combination with some substance in the tubule cells present in constant but limited amount and that subsequent breakdown of this complex allows the transfer of the glucose to the blood (Fig. 15).

Glomerular filtrate	Tubular fluid	Tubule cells	Interstitial fluid	Blood
G →	G →	+A → GA → A+	→ G →	G

Fig. 15. G = glucose; A = substance in tubular cells with which glucose combines.

It is thought that the second reaction proceeds rather slowly in relation to the attainment of equilibrium in the first reaction, namely the combination of G with A to form GA. If the amount of glucose in the glomerular filtrate is increased sufficiently a point occurs when the glucose is proffered to the tubule cells more rapidly than it can be dealt with. As a result glycosuria will occur. In the normal individual this point is not reached under ordinary conditions in the usual ranges of blood-sugar concentration. In the renal glycosuric, however, this mechanism of tubular reabsorption appears to be in some way inefficient and so glycosuria may occur in fasting individuals or in less severe cases after a meal. Friedmann, Selzer, Sugannan & Sokolov (1942) have measured the rate of tubular reabsorption of glucose in normal individuals and in renal glycosurics at different levels of blood-glucose concentration. They were able to demonstrate that in the usual ranges of blood-sugar concentration (100–200 mg. %) the rate of tubular reabsorption was much less than in normals. Curiously enough, however, at much higher and quite unphysiological levels of blood glucose this difference was not apparent.

TABLE 12. *Glucose tolerance tests in normals and renal glycosurics*

Blood sugar in mg. per 100 ml. capillary blood; urine sugar shown by graded reduction of Benedict's qualitative solution. After Houston & Merivale (1949). All these individuals are members of family shown in Fig. 2 (Page 15).

Type of subject	Specimen	Fasting	½ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.
Normal	Blood	75	116	119	90	90	86
	Urine	–	–	–	–	–	–
	Blood	84	149	135	130	87	65
	Urine	–	–	–	–	–	–
	Blood	112	145	119	110	94	102
	Urine	–	–	–	–	–	–
	Blood	61	139	146	130	111	63
	Urine	–	–	–	–	–	–
Glycosuric	Blood	73	100	88	77	47	38
	Urine	–	+ +	+ +	+ +	+	–
	Blood	98	142	173	116	110	n
	Urine	±	+ + +	+ + + +	+ +	–	n
	Blood	84	n	100	63	63	63
	Urine	–	+ + +	+ + +	+ + +	+ + +	n
	Blood	96	126	166	102	78	66
	Urine	±	±	+ +	+	+	±

n = not tested.

The anomaly is specific for glucose. Renal function is in other respects quite normal, and there is no diminution in the tubular reabsorption of other metabolites such as amino-acids. It is now generally agreed that the condition is harmless and asymptomatic. There was, it is true, some discussion as to whether it might not, on occasion, develop into diabetes mellitus. No such development was observed by Marble (1932) in a series of renal glycosurics of the most extreme type who had been observed for a period of 10 years or more. In one of them the condition had been known to exist for 52 years. Similar observations have been made, and the same conclusion arrived at, by Hjarne (1927), Soisalo (1933), and Lawrence (1940).

CYSTINURIA

It has already been pointed out that the term 'cystinuria' comprises more than one quite distinct entity. In the past this group of conditions was always regarded as being due to some 'error' or block in the intermediary metabolism of cystine, leading to the accumulation of this amino-acid in the body and its consequent excretion in large quantities in the urine. The exact nature of this postulated block in cystine metabolism had, however, always been somewhat obscure (Garrod, 1923). The recognition of the different types of 'cystinuria' has, however, made possible a new approach to the problem.

The commonest type is that in which, besides cystine, the urine contains greatly increased amounts of the basic amino-acids lysine and arginine. The other urinary amino-acids are present in normal amounts. All cases of cystine stone formation are probably included in this group, though of course many individuals with this particular abnormality fail to form stones and are quite symptomless. Dent (1949) put forward the view that the anomaly in this class of case is renal in origin. He suggested that in these individuals the reabsorption of the amino-acids, cystine, lysine and arginine, by the renal tubules from the glomerular filtrate, was deficient and that consequently the substances appear in large quantities in the urine. The main direct evidence for this hypothesis rests on the comparison of the concentration of these amino-acids in the blood plasma of cystinurics and of normal individuals. On the view that cystinuria arises from some kind of block in the intermediary metabolism of these substances one might expect that in this condition they would accumulate in the body fluids and that consequently their level in the blood plasma would be considerably higher than in normal people. Thus, in phenylketonuria, a block in the intermediary metabolism of the amino-acid phenylalanine leads to a twenty-fold increase in the concentration of this amino-acid in the blood and cerebrospinal fluid. On the view that cystinuria is a renal peculiarity no such rise in plasma levels of cystine, lysine or arginine would be expected, if anything they might be slightly lowered.

An overall comparison of the plasma amino-acids in normal and cystinuric individuals may be made by chromatography on filter-paper. When this was done the amino-acid pattern in the two groups of people was found to be indistinguishable (Dent, 1949). In particular, the spots representing the amino-acids cystine, lysine and arginine were not obviously more intense or more prominent relative to the other amino-acids in the cystinuric subjects than in the normals. A more exact estimation of cystine in plasma may be made by using the technique of polarography. In a series of eleven normals and nine cystinurics studied in this way (Fowler, Harris & Warren, 1952) the mean concentration of the cystine in the plasma was found to be $0{\cdot}82 \pm 0{\cdot}31$ mg. % in the normals and $0{\cdot}69 \pm 0{\cdot}23$ mg. % in the cystinurics. The cystinurics had in fact a slightly lower cystine concentration, though this difference was not significant in this number of cases. A number of estimations have also been made microbiologically and substantially the same result was found.

Apart from the above observations the actual discovery that both lysine and arginine were as much involved as cystine in this disorder made the earlier ideas of a block in the intermediary metabolism of the latter substance more difficult to maintain. An elaboration of such a hypothesis to take into account these new facts cannot be very readily formulated because of the lack of any obvious connexion between the intermediary metabolism of the basic amino-acids lysine and arginine on the one hand and of cystine on the other.

The renal hypothesis implies that in normal individuals the tubular reabsorption of cystine, lysine and arginine has at least one step which is common to, and specific for, these amino-acids, but it is not involved in the reabsorption of the other amino-acids. No direct evidence for this is forthcoming in man, but experimental work in animals does support the underlying conception that different groups of amino-acids may be absorbed by more or less independent mechanisms (Beyer, Wright, Russo, Skeggs & Shaner, 1947).

With certain modifications it is possible to apply the general theory suggested by Shannon (1939) for the reabsorption of glucose to the reabsorption of amino-acids. One might envisage the peculiarity in such individuals as cystinurics or renal glycosurics as arising from a failure in one or other of the steps involved in reabsorption, due to the absence or relative inefficiency of the particular enzyme concerned in catalysing this step. It might be, on the other hand, that the particular substance in the tubular cells, which it is postulated forms a reversible combination with the reabsorbed amino-acid or sugar, may be present in inadequate amounts due to some failure in its synthesis.

β-AMINO*ISO*BUTYRICACIDURIA

Routine examination of human urines for amino-acids by the technique of paper chromatography has brought to light at least one characteristic pattern of amino-acid excretion which may represent another genetically determined specific peculiarity in renal function. The chromatograms obtained from most urines have a very typical appearance. Glycine, serine, alanine, glutamine, taurine, and often histidine, are readily identified and appear to be present in rather higher concentrations than most of the other amino-acids. Occasionally, however, a substance β-amino*iso*butyric acid (Crumpler, Dent, Harris & Westall, 1951) (Fig. 16), which is probably present in at least traces in all individuals, is found in comparatively large quantities, the spot it gives on the chromatogram being of the same order of intensity or even stronger than the glycine, alanine or glutamine spots, which are usually the most prominent (Fig. 17).

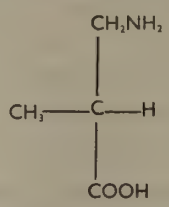
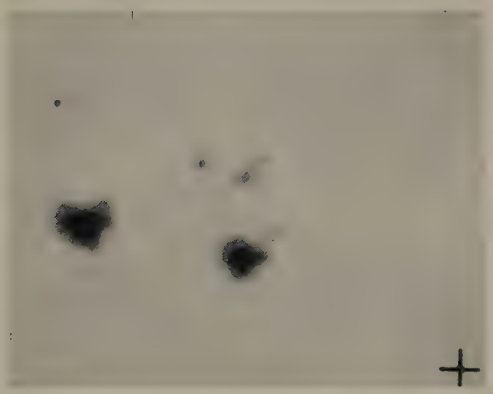


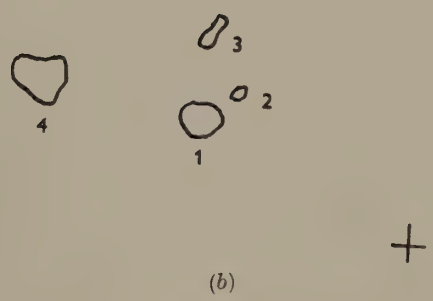
Fig. 16. β-Amino*iso*butyric acid.

Some 5–10 % of normal individuals show this characteristic peculiarity of amino-acid excretion and repeated observations of the same people over months, and in some cases now three or four years, have revealed that the peculiarity persists apparently unchanged. It is equally frequent in both sexes and in all age groups, and is apparently unaffected by ordinary dietary variations. The individuals concerned seem to be perfectly healthy, and no definite association with any morbid condition has been established.

The peculiarity is familial, that is to say, it occurs more frequently among the close relatives of individuals with the trait than in the general population. In view of its evident constancy, the lack of any obvious environmental factors influencing its occurrence, and its familial incidence, it seems probable that it represents a genetically determined variant. The fact that



(*a*)



(*b*)

Fig. 17. Chromatogram of urine from an individual excreting a large amount of β-amino*iso*butyric acid. Chromatogram run in same way as described in Fig. 3. Key: (1) glycine, (2) serine, (3) taurine, (4) β-amino-*iso*butyric acid.

paper chromatograms of the plasma amino-acids in such people reveal no increase in its concentration in the blood, has led to the suggestion that the anomaly is dependent on a specific peculiarity in renal tubular reabsorption.

People showing this peculiarity probably excrete between 100 and 300 mg. of the substance a day. In other people the daily excretion may be of the order of 10–40 mg. There is probably some overlap in the two distributions, and in the absence of a satisfactory method of routine estimation it has not yet been possible to come to any firm conclusions regarding the genetical processes involved.

As yet little is known of the ordinary metabolic role of this new amino-acid.

VI. THE HAEMOGLOBIN VARIANTS

If the idea of genetically determined blocks in intermediary metabolism is correct then we might assume that the particular gene involved is normally concerned with the synthesis of some specific enzyme, and that in the mutant the enzyme is either not formed or is only formed in inadequate amounts or that a variant of the enzyme is produced which, for the particular catalysis we are interested in, is relatively inefficient. A similar conception might well account for some of the examples of genetically determined peculiarities in renal tubular absorption.

Since enzymes are proteins in nature the character of genetically determined variations in protein synthesis may be expected to throw further light on the problem. Two examples where there seems to be reasonable evidence for gene-controlled differences in protein synthesis have already been mentioned. These are the two types of methaemoglobinaemia. In one of these there appears to be a failure to synthesize in its specific form a particular flavoprotein, in the other an abnormal variant of haemoglobin is synthesized. A third example which might be cited is the condition known as afibrinogenaemia (de Silva & Thanabalasundaram, 1951). This is a rare disease, apparently inherited as a typical Mendelian recessive character, and characterized by the complete failure to synthesize the plasma protein fibrinogen. As a result the normal clotting of the blood cannot take place and severe and eventually fatal haemorrhages ensue.

However, perhaps the most instructive variations in protein synthesis that have so far been discovered are concerned with the haemoglobin variants which have, in the last few years, been shown to represent the biochemical basis for the condition known as sickle-cell disease.

SICKLE CELLS

The red blood cells of certain individuals possess the peculiar property of undergoing a reversible alteration in shape in response to changes in the partial pressure of oxygen. When the oxygen tension is lowered these cells change from their normal biconcave form to elongated filamentous and crescentic forms. The peculiar shape of such cells was first recognized in man by Herrick (1910), but what appear to have been substantially the same appearances were described by Gulliver as early as 1840 in some blood films prepared from deer in the London Zoo. The discovery that sickling only occurs under conditions of low oxygen tensions and that when the sickled cells are exposed to sufficient oxygen they revert to their original shape was made by Hahn & Gillespie in 1927.

Most individuals whose erythrocytes can be induced to sickle appear to be perfectly healthy and to suffer no ill-effects from the condition. Occasionally, however, the peculiarity is associated with haemolytic anaemia of varying degrees of severity. The former class are said to possess the sickle-cell trait, the latter to suffer from sickle-cell anaemia. Provided the oxygen tension is reduced sufficiently all the erythrocytes in both classes of individual can be induced to sickle. However, a considerably greater reduction in the partial pressure of oxygen is required for a major fraction of 'trait' cells to sickle than for the 'anaemia' cells (Fig. 18). *In vivo*, some 30–60 % of erythrocytes in the venous circulation of sickle-cell anaemic individuals, but less than 1 % in the trait individuals, are normally sickled (Sherman, 1940). Neel (1951) considers that, using certain techniques, the sickling in the anaemia tends to be more elongate and filamen-

tous than in the trait and this can be used for their differentiation (Fig. 19). Sherman pointed out that, in the anaemic individuals, there must be a cyclic morphological change in a percentage of the erythrocytes with each passage through the systemic and pulmonary circulation.

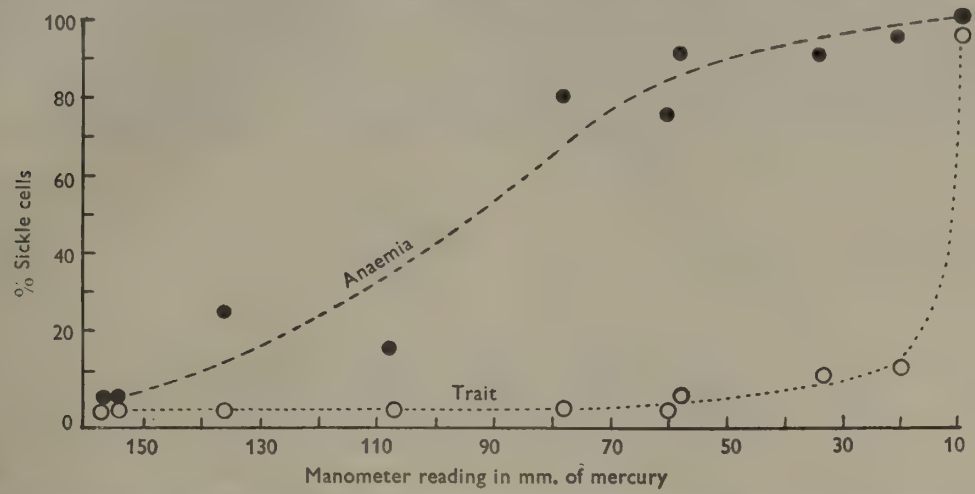


Fig. 18. Production of sickling by reduced atmospheric pressure. Each curve is based upon the total determinations done with the blood of two patients. Each determination was obtained in parallel with a determination on the other curve. (After Sherman, 1940.)

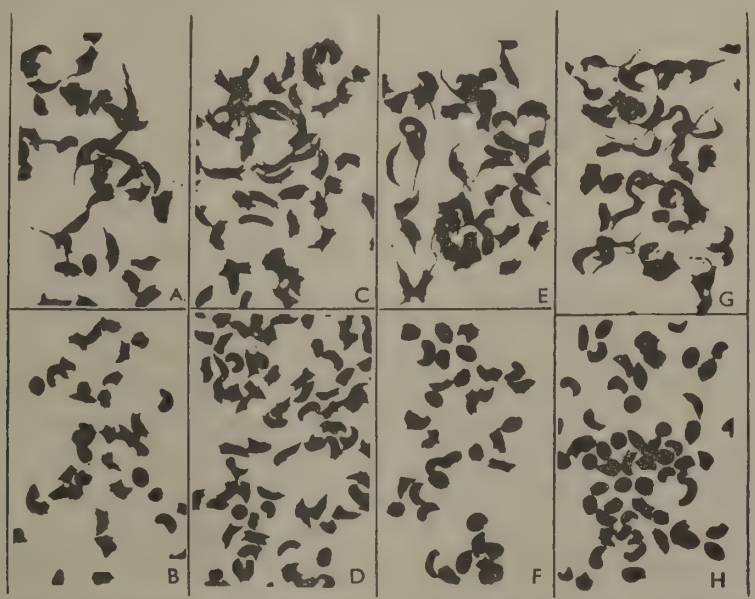


Fig. 19. A comparison of the character of the sickling produced by the Beck & Hertz method in sickle-cell disease and the sickle-cell trait. The upper row of photomicrographs illustrates the filamentous type of sickling observed in four individuals with sickle-cell disease, while the lower row illustrates the type of sickling present in their parents with the sickle-cell trait. (After Neel, 1951.)

The excessive haemolysis in sickle-cell anaemia must be regarded as being due to an intracorpuscular rather than an extracorpuscular defect. Thus the trait cells behave like normal cells when transfused into the circulation of patients with sickle-cell anaemia, and survive for approximately 120 days. Sickle-cell anaemic cells, however, consistently show a markedly shortened life span (Callender, Nickel, Moore & Powell, 1949; Singer, Robin, King & Jefferson, 1948).

INCIDENCE AND GENETICS

The sickle-cell trait is common among Negro populations in Africa and the United States. Of all Africans living south of the Sahara some 15 % are believed to possess this anomaly, and among Negroes in the United States the frequency is of the order of 9 %. Apart from these populations an appreciable incidence has been reported among Yemenite Jews, in certain populations in South India, and in scattered groups in Greece. It appears to be extremely rare among white Americans and in Europe.

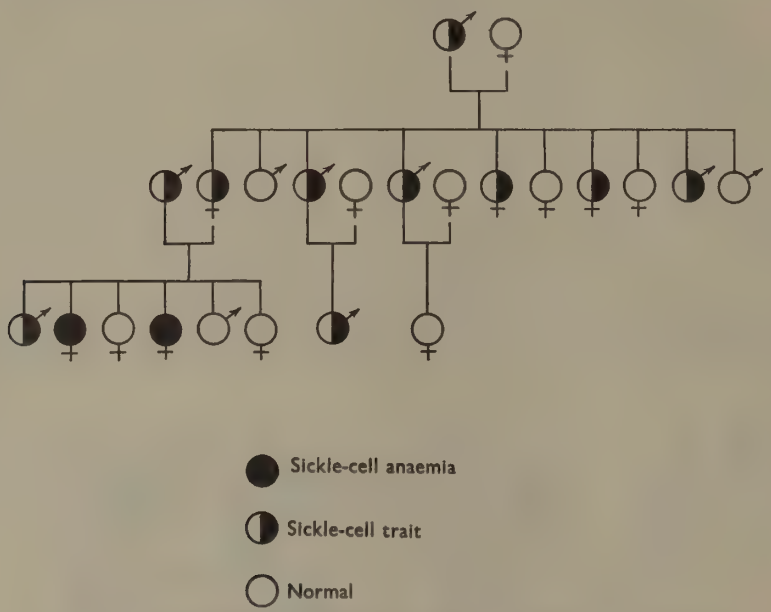


Fig. 20. Pedigree of sickle-cell disease. (After Neel, 1951.)

The hereditary nature of the condition was first recognized by Emmel in 1917. Taliaferro & Huck (1923), from a study of a large Negro family containing many individuals with the sickle-cell trait, came to the conclusion that the condition was inherited as a simple Mendelian dominant character. At this time little distinction was made, from the genetical point of view, between the asymptomatic individuals with the 'trait' and those with frank haemolytic anaemia. Both were known to occur together in the same families and it was assumed that the condition produced by the abnormal gene in heterozygous form was extremely variable and the two types of case simply represented extremes of a continuous distribution. This view has been widely accepted until quite recently when, more or less simultaneously, Neel (1949) and Beet (1949) put forward the hypothesis that the sickle-cell trait and the sickle-cell anaemia occurred respectively in individuals heterozygous and homozygous for the same abnormal gene. The trait individuals possessed in their genetical constitution only a single dose of the abnormal gene, the 'anaemic' individuals had a double dose. Neel (1951) has now collected a large body of familial data which, for the most part, afford strong evidence for this hypothesis.

In the first place he has found that, in the main, individuals with sickle-cell anaemia can, on clinical and haematological grounds, be sharply distinguished from those with sickle-cell trait. Discriminative criteria based on the presence of haemolytic anaemia, the presence of evidence

of increased haemolysis (elevated serum bilrubin) and regeneration (reticulocytosis), the occurrence of leucocytosis, and the clinical history and physical findings, left little doubt as to the classification of most individuals into one or other category. Subsequent work on the biochemical nature of this condition has since revealed the underlying nature of the occasional anomalous cases not readily classifiable by the above methods. Having made this initial classification, Neel then proceeded to examine the manner in which the two classes of individual were distributed in a series of some seventy-five different families.

The hypothesis that the sickle-cell trait and the sickle-cell anaemia are related as heterozygote and homozygote requires that both parents of all sickle-cell anaemic individuals should show the trait; that the incidence of sickle-cell anaemia in sibships segregating for this condition, and where neither parent was a sickle-cell anaemic, should be 0·25; that, among the sibs of sickle-cell anaemics, the trait should be twice as common as the anaemia; that the offspring of matings between normal individuals and individuals with the trait should be, on the average, half normal and half with the trait; and that the offspring of parents one of whom has sickle-cell anaemia and one of whom is normal should all show the sickle-cell trait. With few exceptions the distribution of the trait and the anaemia among the seventy-five different families studied by Neel fell into this pattern. Thus, of ninety-four parents of sickle-cell anaemic children who could be examined, ninety-three showed the sickle-cell trait. The incidence of sickle-cell anaemia in sibships segregating for this condition was estimated to be between 0·18 and 0·26. Among the sibs of sickle-cell anaemic individuals the ratio of sibs with the trait to sibs with the anaemia was 60 : 34. Of the four children who were offspring of a sickle-cell anaemic parent all showed the trait. The main exception arose in sibships resulting from the marriage of normal with sickle-cell trait individuals. Here the observed ratio of those with the trait to the normals was somewhat less than the expected 1 : 1.

In spite of the exceptions the general theory suggested by Neel appears to be the most reasonable explanation of the observed distribution. Several causes might lead to the occurrence of exceptions to the expected pattern. Illegitimacy could produce the occasional case where one parent of a sickle-cell anaemic was found to be normal or where both parents of a sickle-cell trait individual failed to show the trait. The occurrence of a mutation might lead to the same result. An apparently normal parent could contribute a sickle-cell gene to one of his offspring in consequence of a mutation occurring at some stage during gametogenesis. Differential mortality among the different types of individual has also to be considered as a possibility leading to distorted ratios. A final possible cause of anomalous findings is the occurrence of haemolytic anaemia resulting from the interaction of the sickle-cell gene with some other abnormal gene either at the same or a different locus. In fact, subsequent studies of the biochemical background of this condition have demonstrated the occasional occurrence of precisely this phenomenon.

SICKLE-CELL HAEMOGLOBIN

Pauling, Itano, Singer & Wells (1949) made the fundamental discovery that the erythrocytes of patients with sickle-cell anaemia contained haemoglobin having a significantly different isoelectric point from haemoglobin derived from normal individuals. It was found that the electrophoretic mobilities of both the ferrohaemoglobins and the carbonmonoxy derivatives differed over quite a wide range of pH. For example, at pH 6·9 in phosphate buffer the

carbonmonoxy haemoglobin from sickle-cell anaemic individuals migrated as a positive ion and the normal derivative as a negative ion (Fig. 21 and Table 13).

In individuals with the sickle-cell trait, a mixture of the two types of haemoglobin was found. Approximately 60% being of the normal and 40% of the abnormal type. Since all the erythrocytes in such individuals were known to sickle provided the oxygen tension was sufficiently low, it appeared that both types of haemoglobin were probably present in each cell.

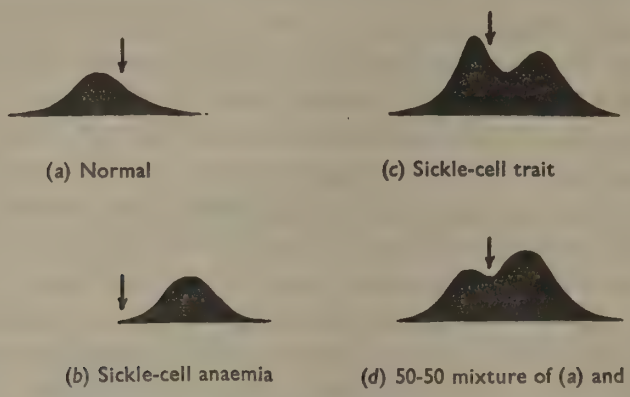


Fig. 21. Electrophoretic diagrams of carbonmonoxyhaemoglobins in phosphate buffer pH 6·9. (After Pauling *et al.* 1949.)

TABLE 13. *Isoelectric points of normal and sickle-cell haemoglobin*

Compound	Isoelectric points in phosphate buffer		
	Normal	Sickle-cell anaemia	Difference
Carbonmonoxy haemoglobin	6·87	7·09	0·22
Ferrohaemoglobin	6·68	6·91	0·23

Pauling and his colleagues pointed out that these findings were readily interpreted in terms of Neel's genetical hypothesis that sickle-cell anaemia and sickle-cell trait occurred respectively in individuals homozygous and heterozygous for the sickle-cell gene. The normal allele of this gene could be regarded as controlling more or less directly some step in the synthesis of haemoglobin. The sickle-cell gene leads to some peculiarity in this process, resulting in the formation of an abnormal haemoglobin. In the heterozygote both lines of synthesis proceed, the former being somewhat more efficient. A direct quantitative relationship between the genes present and the haemoglobins formed appeared to exist.

Perutz & Mitchison (1950) demonstrated a second important characteristic of this new and abnormal type of haemoglobin. They found that the reduced sickle-cell haemoglobin was considerably less soluble than reduced normal haemoglobin (Fig. 22). The oxyhaemoglobin derived from sickle-cell anaemic individuals, on the other hand, appeared to be equally soluble as that derived from normals. Their experiments were conducted at pH 6·8 in concentrated phosphate buffer. However, Perutz, Liquori & Eirich (1951) showed that the same solubility relationships held under conditions of salt concentration, pH and temperature normally encountered in the red blood cell. These observations suggested a direct explanation of the

phenomenon of sickling which occurs when erythrocytes containing the abnormal haemoglobin are exposed to an atmosphere of low oxygen tension. The reduced haemoglobin would be expected to crystallize and the sickling to result from the consequent deformation of the red cells, partly by the 'habit' of the crystals and partly by the loss of water which would be expected to accompany crystallization. While the erythrocytes contain some 34% haemoglobin, the crystals contain some 50–60%. The surplus water not enclosed in the crystals would tend to

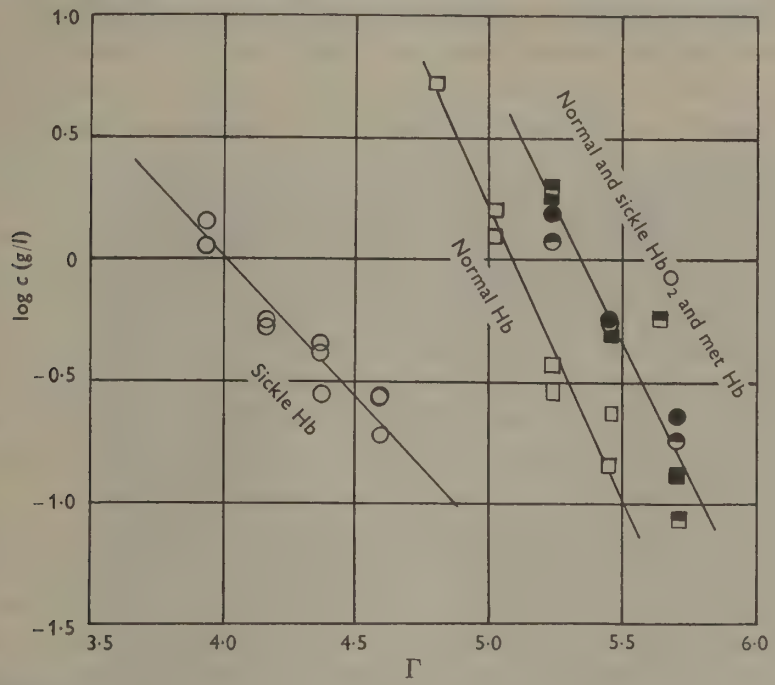


Fig. 22. Solubilities of normal and sickle haemoglobins plotted against ionic strength of phosphate buffer. ○, sickle haemoglobin; ●, sickle oxyhaemoglobin; ◓, sickle methaemoglobin; □, normal haemoglobin; ■, normal oxyhaemoglobin; ⬒, normal methaemoglobin. (After Perutz & Mitchison, 1950.)

diffuse out of the cell through loss in osmotic pressure, thus causing the cell membrane to collapse. The bi-refringence of sickled cells first observed by Sherman (1940) could also be explained by the idea that they contained crystalline-reduced haemoglobin. The solubility of totally reduced sickle-cell haemoglobin was so low that it appeared unlikely that more than one-seventh of that normally present in the red cell could be present in solution. Though a certain degree of supersaturation might be expected, it seemed likely that the reduced haemoglobin would begin to solidify long before the cell became completely deoxygenated. Where a mixture of normal and abnormal haemoglobin was present sickling would only be expected at relatively lower oxygen tensions. Thus the characteristic differences in behaviour between 'trait' and 'anaemic' cells could be largely explained.

Substantially similar conclusions had been arrived at by Harris (1950) who showed that sickle-cell haemoglobin solutions, free from any cell stroma, became increasingly viscous as the oxygen tension was decreased and that the more concentrated solutions assumed a semi-solid gel-like state. Microscopically, spindle-shaped bodies 1–15μ in length could be observed, and these when examined under the polarizing microscope were bi-refringent. They showed all the characteristics of tactoids. They disappeared on reoxygenation of the solution and reformed

again when the oxygen was removed. There were remarkable similarities in shape between sickled cells and these haemoglobin tactoids formed in these stroma-free solutions of deoxygenated sickle-cell haemoglobin (Fig. 23).

THE NATURE OF THE ABNORMAL HAEMOGLOBIN

As yet, attempts to elucidate the structural difference between the normal and sickle-cell haemoglobin have been unsuccessful. Pauling and his colleagues concluded that the sickle-cell anaemia haemoglobin had two to four more net positive charges per molecule than normal haemoglobin. Samples of the porphyrin dimethyl esters prepared from normal and sickle-cell haemoglobin were found to be identical on the basis of the X-ray powder photographs and the identity of the melting-points and mixed melting-point. It was, therefore, concluded that the difference must lie in the globins. Amino-acid analysis of these revealed no difference with respect to their content of basic or acidic amino-acids (Schroeder, Kay & Wells, 1950). However, there was some evidence that the sickle-cell haemoglobin contained slightly less leucine and valine, and slightly more serine and threonine than the normal. It was thought that although the amino-acids in which the proteins seemingly differed could not in themselves contribute directly to the difference in the net charge on the proteins and hence influence the electrophoretic properties, they might possibly affect the folding or coiling of the polypeptide chains, and in this way either bring into play or remove from action charged groups, thus indirectly altering the electrophoretic behaviour.

X-ray analysis of crystals of sickle-cell and normal haemoglobin were made by Perutz *et al.* (1950). Much to their surprise, it was found that the X-ray diagrams obtained from the two different proteins were identical in every detail. The authors remark 'complete identity of the X-ray diffraction patterns could never occur if any gross differences existed between the chemical structure of the polypeptide chains or their number or arrangement in the molecules. To give identical unit cell dimensions, the dimensions of the molecules must be identical and the structural differences must be so located that they do not influence the intermolecular forces in the particular crystal lattice. To give the same relative X-ray intensities, the differences must be such that they do not alter the mass distribution in more than a small fraction of the molecule.' Thus the findings give no clue as to the structural differences of the molecule but merely serve to emphasize their close similarity.

THE PROPORTION OF SICKLE-CELL HAEMOGLOBIN TO NORMAL HAEMOGLOBIN IN THE SICKLE-CELL TRAIT

In their first investigation of this problem Pauling and his colleagues had observed that rather less than half the haemoglobin in the heterozygous individuals with the sickle-cell trait was of the abnormal type. It soon became clear that the actual proportion found in different cases was very variable. Wells & Itano (1951) found, in forty-two unrelated sickle-cell trait individuals, percentages of abnormal haemoglobin varying from 24 to 45 % with an arithmetic mean of 37·5 %. The distribution showed some tendency to bimodality with one mode at 34–36 % and the other at 40–42 %. The actual error in any one determination was probably not more than $\pm$ 1·5 %, and repeated samples from the same individual gave values which were substantially constant. Age appeared to have little or no influence on the proportion, and there was very little difference between the two sexes. The proportions found in husbands and wives were not

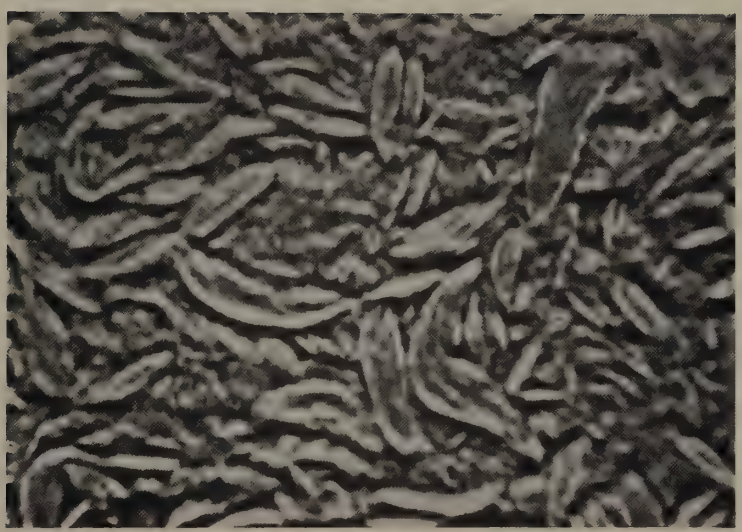


Fig. 23. Tactoids in stroma-free solutions of reduced sickle-cell haemoglobin. (Phase microscopy, × 1400). (After Singer *et al.* 1952.)

TABLE 14. *Percentages of sickle-cell haemoglobin among individuals with the sickle-cell trait in different families*

After Neel *et al.* 1951.

Family	Percentage of sickle-cell haemoglobin	
	Parent with sickle-cell trait	Children with sickle-cell trait
1	34·0	34·5
		35·5
2	36·8	43·0
3	27·7	35·5
		22·3
		36·2
4	45·2	43·6
		42·2
		45·2
		44·2
5	41·0	41·0
		44·0
		42·5
		43·0
6	40·9	31·9
		31·1
		34·5
		31·5
7	34·4	34·2
		34·7
		40·7
		40·9
		41·5
		31·8
		40·7

Source of variation	Analysis of variance		
	Degrees of freedom	Sums of squares	Mean square
All determinations	31	972·52	.
Between family means	6	635·73	105·96
Between members of same family	25	336·79	13·47

Variance ratio, $F = 105{\cdot}96/13{\cdot}47 = 7{\cdot}87$. $P < 1\%$.

significantly correlated, nor were there any obvious differences in individuals coming from different geographical regions of the United States. Thus the variation could not be very easily accounted for in terms of any gross environmental differences. It appears, in fact, that much of the variation is genetically determined. In a series of families segregating for the sickle-cell trait (Table 14) Neel, Wells & Itano (1951) found that, with respect to the proportion of abnormal haemoglobin in the heterozygous individuals, the variation between the families was

significantly greater than the variation within the families. In other words, in some families the average percentage of abnormal haemoglobin was significantly greater than in others. The exact interpretation of this finding has not yet been worked out. The possibility that there were more than one sickle-cell gene, each producing somewhat different proportions of abnormal haemoglobin in the heterozygotes, could not be readily sustained on detailed examination of the pedigrees, because both high and low values could be found together in certain families. One must assume that there exist other genes ('modifiers') segregating in the American Negro population, capable of influencing significantly the proportion of haemoglobin present in individuals heterozygous for the sickling gene.

FOETAL HAEMOGLOBIN IN SICKLE-CELL ANAEMIA

That the situation was not going to prove as simple as had at first been envisaged was emphasized by the finding of Singer, Chernoff & Singer (1951*a*, *b*) and Singer & Chernoff (1952) that, in sickle-cell anaemia corpuscles, but not in the sickle-cell trait corpuscles there existed very frequently an appreciable amount of what appeared to be foetal haemoglobin. Ever since the work of von Körber (1866), it has been known that the haemoglobin of the foetus is different from that found in the adult. The most convenient method of differentiating the two types in man is based on the fact that here foetal haemoglobin is much more resistant to alkali denaturation than is adult haemoglobin. This difference may in fact be made the basis of a quantitative method for determining the proportion of the two haemoglobins present in any mixture. Foetal and adult haemoglobin can be shown to differ in other respects. They have a different ultra-violet spectral absorption, they are immunologically different, and their crystalline derivatives belong to different systems. In the newborn infant some 80–90 % of the circulating haemoglobin is of the foetal type. With the development of the infant the foetal type gradually disappears and, by the end of the first year all, or nearly all, of it is replaced by the adult type.

Singer *et al.* (1951*a*, *b*), using the method of alkali denaturation, found that, in forty-five typical cases of sickle-cell anaemia, irrespective of age, 2–24 % of pigment was alkali resistant and presumably foetal in type. In ninety cases of the sickle-cell trait, foetal-like haemoglobin was entirely absent.

	Haemoglobin		
	Normal adult	Sickle cell	Foetal
Sickle-cell anaemia	Absent	76–98 %	2–24 %
Sickle-cell trait	55–77 %	23–45 %	Absent
Normal	100 %	Absent	Absent

Now, although Pauling and his colleagues had originally thought that, in sickle-cell anaemia, all the haemoglobin was of the abnormal type, this view has had to be modified as the material was extended. Wells & Itano (1951) found that in some cases of sickle-cell anaemia from 5 to 20 % of a pigment behaving electrophoretically like normal haemoglobin and clearly distinguishable from the abnormal haemoglobin could be demonstrated. Smaller amounts of this material might well in other cases have been obscured in the electrophoretic separation by the proportionately much larger and overlapping abnormal haemoglobin present. It seems

likely that the foetal-like haemoglobin observed by Singer *et al.* (1951*a*, *b*) corresponds to this 'normal' component found by Wells & Itano (1951). Thus the difference between the normal sickle-cell trait and sickle-cell anaemic individuals with respect to the three different haemoglobins thus far discovered could be represented as shown on p. 54. Reduced foetal haemoglobin derived from cord blood, and reduced foetal-like haemoglobin obtained from sickle-cell anaemic individuals resembled normal adult-reduced haemoglobin in failing to show tactoid or gel formation on concentration. In this respect they differed from sickle-cell haemoglobin.

FURTHER TYPES OF HAEMOGLOBIN

The study of families in which apparent exceptions to the general pattern of inheritance had been observed led to the discovery of two further types of haemoglobin.

Itano & Neel (1950) examined electrophoretically the haemoglobins derived from members of two such families. In these families there occurred one or more children with a haematological picture of sickle-cell anaemia, but the disease was somewhat less severe than that usually encountered and the situation was peculiar in that the erythrocytes of only one parent in each case could be induced to sickle. The other was apparently normal. Electrophoretically it was found that the haemoglobin from two of these anaemic children separated into two distinct components (Fig. 24); one of these corresponded to that of sickle-cell haemoglobin, the other migrated as an even more positive ion than sickle-cell haemoglobin. In the other anaemic child both these components were present as well as a small proportion of haemoglobin with a mobility corresponding to normal haemoglobin. Studies of haemoglobin samples from the other members of the families showed that in each case the haemoglobin of the parent whose erythrocytes sickled showed the findings typical of sickle-cell trait, that is, a mixture of normal and sickle-cell haemoglobin, with the former predominating. The other parent (non-sickling) was shown in each case to have haemoglobins resolving into two types, the normal and the new component, with mobility faster than either sickle-cell haemoglobin or normal. One child in each family had 100% normal haemoglobin and another had a mixture of the new type of haemoglobin and of normal.

Mobility of normal, sickle cell, and the new haemoglobin in cacodylate sod. chloride buffer pH 6·5, *ionic strength* 0·1

After Itano & Neel (1950).

	cm.sec./V./cm.^{2}
Normal	$2{\cdot}4 \times 10^{-5}$
Sickle cell	$2{\cdot}9 \times 10^{-5}$
New haemoglobin	$3{\cdot}2 \times 10^{-5}$

The findings suggested that the tendency to form this new type of haemoglobin was inherited as if due to a single dominant gene. Whether such a gene was allelic to the sickle-cell gene or at some other locus could not be determined from the above data. The haematological findings in the individuals who presumably received both abnormal genes, one from each parent, could be accounted for either on the basis of factor interaction on the part of two independent genes at different loci, or as a consequence of multiple allelism. The problem can ultimately be resolved by the study of the offspring of such individuals when they marry normals. If the allelic hypothesis is true only two types of offspring will result, one with normal and sickle-cell haemoglobin,

the other with normal and the new haemoglobin. If the genes are at different loci not closely linked, four different types of offspring may be expected, these would have (*a*) all normal haemoglobin, (*b*) normal and sickle-cell haemoglobin, (*c*) normal and the new haemoglobin, (*d*) sickle cell and the new haemoglobin and perhaps some normal haemoglobin.

Clinically the compound heterozygotes (i.e. the individuals with a mixture of both sickle cell and the new haemoglobin) represented a syndrome intermediate between the benign sickle-cell trait and sickle-cell anaemia. There was a mild anaemia with associated splenomegaly. The survival time of erythrocytes when transferred into normals was distinctly shortened. The heterozygous individuals possessing in their erythrocytes a mixture of normal and the new type of haemoglobin were completely asymptomatic. Their erythrocytes did not sickle, but showed some tendency to the target cell deformity and had increased resistance to hypotonic saline. Curiously enough these cells were found to have a distinctly shortened life span after transfusion into normal individuals (Kaplan, Zuelzer & Neel, 1951).

Itano (1951), in the study of another anomalous family in which two children appeared to have sickle-cell anaemia, but one parent failed to show the trait, was able to uncover the presence of a further type of gene-controlled abnormal haemoglobin. He found that electrophoretically the haemoglobin of the anaemic children corresponded closely to that generally found in sickle-cell anaemia. However, in the mother and two sibs, none of whom showed the sickle-cell trait, there were nevertheless two electrophoretically different components in their haemoglobin. Respectively, 42, 35 and 49% of their haemoglobins were found to migrate as sickle-cell haemoglobin and the rest as normal. Thus electrophoretically they could be regarded as sickle-cell heterozygotes even though they failed to show the sickling phenomenon. The explanation of this anomalous result was found as a result of solubility studies. It emerged that the abnormal haemoglobin present in the anomalous individuals in this family, while possessing the same electrophoretic mobility as sickle-cell haemoglobin, could be distinguished clearly from this by the fact that its solubility in the reduced form was considerably greater than reduced sickle-cell haemoglobin and approximated to that of reduced normal haemoglobin. It represented in fact a new molecular species of haemoglobin. The two anaemic children presumably had in their red cells both the sickle-cell type and this new variant.

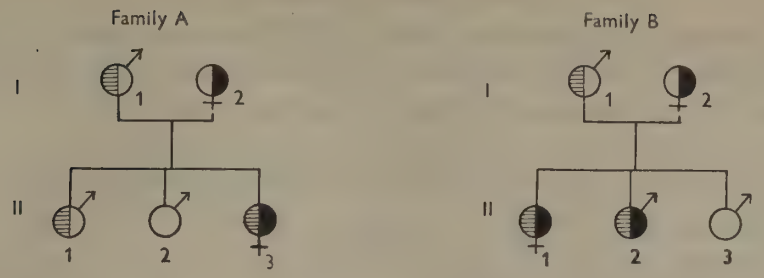
SUMMARY OF THE HAEMOGLOBINS IN SICKLE-CELL DISEASE

It is now possible to identify on the basis of the multiple criteria provided by the techniques of electrophoresis, solubility studies, alkali denaturation, and direct tests for sickling on the erythrocytes, five distinct types of human haemoglobin which it is necessary to consider in connexion with sickle-cell disease. They are summarized in Table 15.

TABLE 15. *Different types of human haemoglobin*

Name	Identification symbol	Causes sickling	Electrophoretic mobility at pH 6·5	Solubility	Resistance to alkali denaturation
Foetal	*f*	No	Slow	High	Resistant
Normal adult	*a*	No	Slow	High	Non-resistant
Sickle cell	*b*	Yes	Intermediate	Low	Non-resistant
	c	No	Fast	High	?
	d	No	Intermediate	High	?

(*a*) Pedigrees of families A and B.



Family		Age	Sickling test	Pattern (see Fig. 24*b*)	Haemoglobin components		
					Normal	Sickle	New component
A I	1	29	−	*d*	64·7	.	35·3
	2	28	+	*c*	66·5	33·5	.
A II	1	6	−	*d*	66·4	.	33·6
	2	4	−	*a*	100·0	.	.
	3	3	+	*f*	13·0	39·0	48·0
B I	1	33	−	*d*	69·8	.	30·2
	2	31	+	*c*	68·9	31·1	.
B II	1	12	+	*e*	.	47·0	53·0
	2	10	+	*e*	.	50·0	50·0
	3	8	−	*a*	100·0	.	.

(*b*) Electrophoretic diagrams of carbonmonoxyhaemoglobins (in cacodylate buffer pH 6·5) from individuals in these families compared to the diagrams obtained from individuals known to be haematologically normal or to have sickle-cell anaemia or sickle-cell trait:

(*a*) Normal.
(*b*) Sickle-cell anaemia.
(*c*) Sickle-cell trait.
(*d*) Family A I 1 and II 1. Family B I 1.
(*e*) Family B II 1 and II 2.
(*f*) Family A II 3.
(*g*) Mixture of *b* and *d*.
(*h*) Mixture of *a* and *e*.

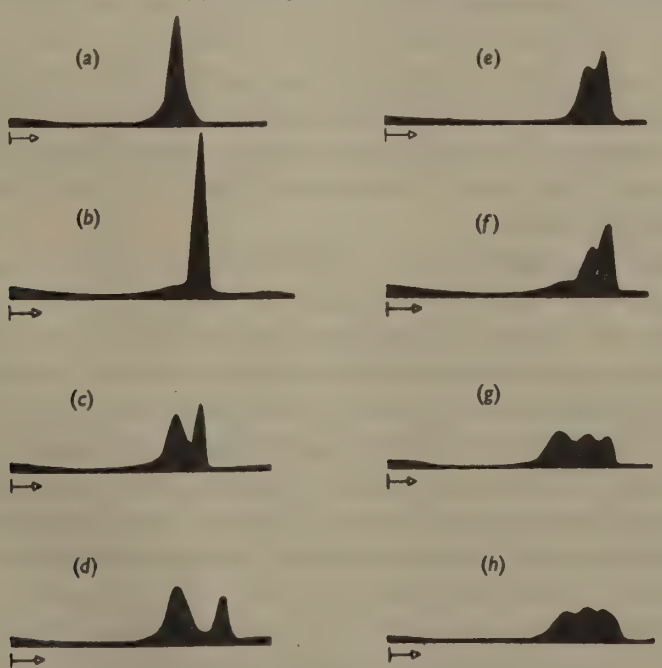
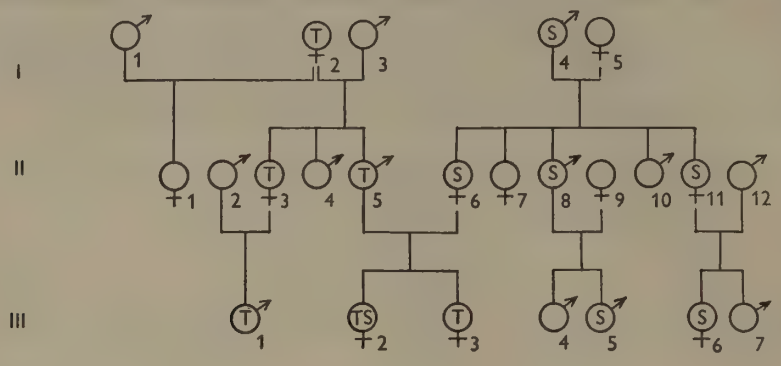
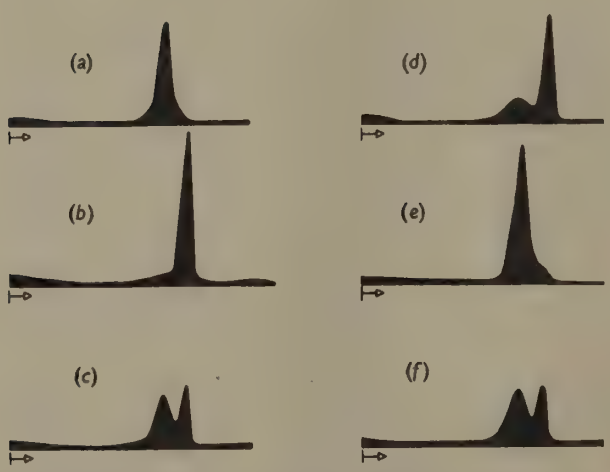


Fig. 24. Families described by Itano & Neel (1950).

sickle-cell trait and thalassaemia minor. The latter is a hereditary haematological abnormality frequently found in Mediterranean countries. It is believed to be determined by a gene which, in homozygous form, produces a severe anaemia and splenomegaly associated with characteristic haematological appearances, notably an increased number of 'target' and oval red blood cells,



(A)



(B)

Fig. 25. Family segregating for both sickle-cell trait and thalassaemia. (A) Pedigree. (B) Electrophoretic diagrams of carbonmonoxyhaemoglobin (pH 6·5): (*a*) normal; (*b*) sickle-cell anaemia; (*c*) sickle-cell trait; (*d*) haemoglobin from III 2; (*e*) haemoglobin from II 5; (*f*) haemoglobin from II 6. (After Sturgeon, Itano & Valentine, 1952.)

peripheral erythroblastosis, and a decreased red-cell fragility. This is called Cooley's anaemia or thalassaemia major. In heterozygous form haematological peculiarities may occur, such as an increased proportion of target and oval cells and a decreased red-cell fragility, but severe anaemia is unusual. This is called thalassaemia minor. Whether or not a specific abnormal

haemoglobin is formed in this disease is not known, but there is evidence that, at any rate in the anaemic cases, a considerable proportion of foetal-like haemoglobin may be formed (Liquori, 1951; Rich, 1952). A number of families have now been described in which one parent had thalassaemia minor (i.e. was heterozygous for the thalassaemia gene) and the other had the sickle-cell trait and was heterozygous for the sickle-cell gene. In each family one of the offspring was found to have a rather atypical anaemia resembling in certain respects both thalassaemia and sickle-cell anaemia. These children are presumably compound heterozygotes and in one case electrophoretic studies have been made on the haemoglobin present (Fig. 25). It was found that two components were present. One moved with the mobility of sickle-cell haemoglobin, and this accounted for 70 % of the total pigment, the other had a mobility indistinguishable from normal haemoglobin and represented only 30 % of the total. Only one component indistinguishable from normal haemoglobin was present in the father, and the mother, who had typical sickle-cell trait, had 38 % of sickle-cell haemoglobin. Thus it seems that at least in this one case a heterozygote for the sickle-cell gene produced as much as 70 % of the abnormal haemoglobin. This situation differs from that in sickle-cell trait, where usually much less of the abnormal haemoglobin is formed, and sickle-cell anaemia, where rather more occurs. The difference presumably arises from the presence of the thalassaemia gene. Evidently some complex interaction is occurring. It is not known for certain yet whether the thalassaemia gene and the sickle-cell gene are allelomorphic or at different loci, but probably the latter is the case (Silvestroni & Bianco, 1952).

VII. THE BLOOD-GROUP SUBSTANCES

Studies on the nature of the blood-group substances and the specific character of the products determined by different genes at the same locus, and also by genes at different loci, depend on the possibility of isolating these compounds in a pure form and characterizing them chemically. This possibility has only been realized as yet in the case of some of the substances responsible for the specific character of the *ABO* blood-group system and the Lewis system. These systems of blood-group antigens have been found particularly favourable material for this kind of investigation because the substances occur not only in the red blood cells but are also widely distributed in the tissues and in the body fluids. It is from the latter that it has been found possible to obtain material in sufficient quantities for detailed analysis.

SECRETORS AND NON-SECRETORS

The specific substances may be identified in tissue extracts by the ability of such extracts to absorb specifically the corresponding antibody. Nearly all the tissues in the body have been shown to possess such specific activity. In a similar manner the presence of these substances has been identified in such body fluids and secretions as saliva, tears, sweat, digestive juices, bile, milk, pleural pericardial and peritoneal fluids, and amniotic fluid.

The striking fact was discovered (Lehrs, 1930; Putkonen, 1930) that certain individuals of groups *A*, *B* and *AB* contained group-specific substances in their saliva in concentrations much higher than occurred in their red blood cells, whereas in other people of the same blood types these substances were completely absent in the saliva. These two classes of individuals have been called 'secretors' and 'non-secretors', and it was found that the ability or inability to secrete the group-specific substances in the saliva was a constant trait in any one individual, and that when the substances were absent from the saliva they were also absent from the other secretions.

It is possible to extract the specific substances from the tissues in two forms: one soluble in organic solvents such as chloroform or alcohol, and the other soluble in water. Friedenreich & Hartmann (1938) found that the alcohol soluble form was present in nearly all the organs of both secretors and non-secretors. The water soluble form could only be extracted from the tissues of secretors. The material in the red cells is present in the alcohol soluble form. The relative concentrations of the substances present in the tissues and secretions is indicated in Table 18 and in Fig. 26.

TABLE 18. *Relative concentration of group-specific substances (ABH) in various body fluids*

After Putkonen (1930).

Saliva	128–1024
Semen	128–1024
Amniotic fluid	64–256
Tears	8–32
Urine	2–4
Cerebrospinal fluid	0

Schiff & Sasaki (1932) showed that the two classes, secretors and non-secretors, were genetically determined. In European populations some 20–25 % of the population fall into the non-secretor class. The familial distributions could be readily understood on the hypothesis that the non-secretors were homozygous for a recessive gene, the dominant allele of which, when present in either homozygous or heterozygous form, determined the character 'secretor'. These two characters appeared to be inherited completely independently from the *ABO* blood groups, and so it was concluded that the two series of allelic genes occur at loci either on different chromosomes, or well separated on the same chromosome. The presence or absence in an individual of either *A* or *B* substance in the saliva could therefore be regarded as being directly dependent on two distinct genes at different loci.

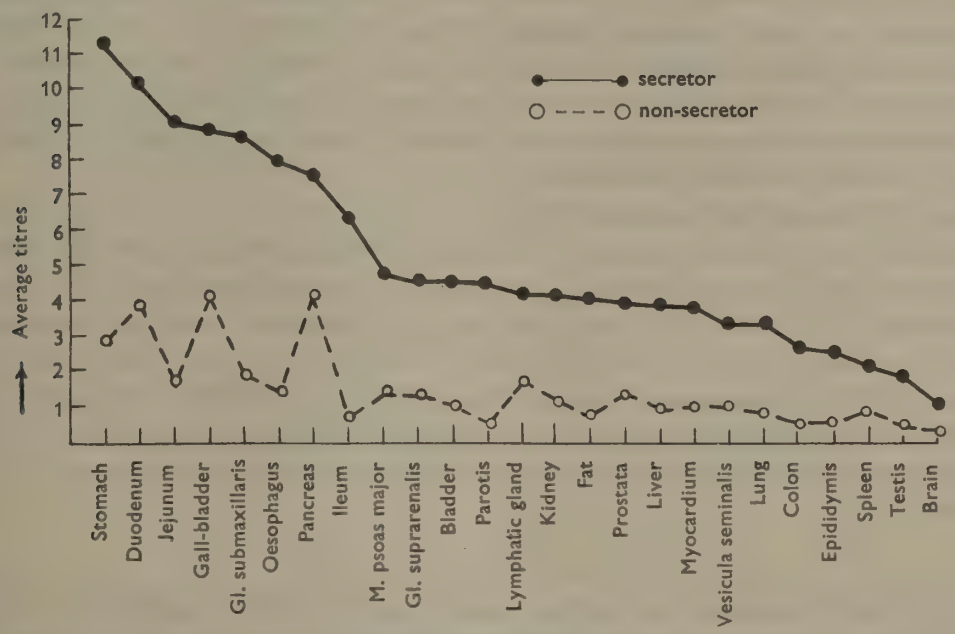


Fig. 26. Average antigen concentration in twenty-five organs of the human body. (After Hartmann, 1941.)

O AND *H*

The original classification into the four blood groups, *A*, *B*, *AB* and *O*, was based on the presence or absence of the agglutinable substances *A* and *B* on the red blood cells. Group *O* erythrocytes were identified as cells devoid of either of these two antigens. The question naturally arose as to whether the group *O* represented simply an absence of *A* and *B* or whether there existed an *O* substance for which, as yet, no specific antiserum had been discovered.

Schiff (1927) first demonstrated that certain carefully selected and absorbed sera from normal cattle would give rise to the agglutination of *O* cells. Subsequently, it was discovered that certain persons of group *O* (secretors) did, in fact, secrete a substance in their saliva which was able to neutralize the agglutinating action of the absorbed cattle sera on *O* red cells. It was therefore thought that such sera could be regarded as true anti-*O* sera reacting with a specific substance *O* produced by Bernstein's gene **O** and secreted in the saliva of secretors of group *O*. A number of sera with similar types of properties were subsequently discovered from a series

of very diverse sources, such as sheep, pigs, cats, dogs, chickens and eels (Hirszfeld & Halber, 1928; Dahr, 1939; Jonsson, 1944). Occasionally such sera, though usually of weak titre, have been found occurring in humans of blood groups A_1B, A_1, or B. Similar sera have also been obtained by the active immunization of rabbits with material from blood group O corpuscles (Hooker & Anderson, 1921) or of substances isolated from ovarian cysts of women of blood group O (Morgan & Waddell, 1945). The same kind of properties have been obtained in sera arising from the immunization of goats and chickens with Shiga bacilli.

The exact nature of the specific character of these sera, however, still remains obscure. If they were specific for a substance O one would expect that not merely red cells from O individuals would be agglutinated, but also the heterozygotes **AO** and **BO**, who would form quite a proportion of individuals falling into the classes A and B. On the other hand, the red cells of AB people should not react. Extensive studies on these various sera have in general failed to demonstrate the simple pattern of reactions anticipated on this hypothesis (Hirszfeld, 1947; Moureau, 1946). In particular, the red cells of AB individuals have often been found to be agglutinated and, furthermore, the saliva of such individuals may contain substances which inhibit the activity of most of these sera.

Morgan & Watkins (1948) concluded that, in the majority of cases, these sera were active against a substance which they called H (to indicate its heterogenetic origin) and which could not be regarded as the specific product of the **O** gene. It was still considered that a substance corresponding to O does occur in O cells, and that occasionally sera occur which are specific for this substance, but in general it is not present in the secretions of O individuals, or in the secretions of individuals heterozygous for O, when they are secretors. The amount of H substance present in the red cells is not thought to be independent of the presence of the other antigens, the agglutinability of red cells of different groups with anti-H being roughly of the order of $O > A_2 > B > A_1$.

THE LEWIS BLOOD GROUPS

In 1946, Mourant found a new antibody in certain samples of human sera, which agglutinated red cells from about 25% of English people, and which was independent of the other known blood group antigenic systems. The familial distribution of positive reactors to sera containing this antibody is readily interpreted on the hypothesis that the potentiality of giving a positive reaction is inherited as a simple recessive character (Andresen, 1947) (Table 19). The main

TABLE 19. *The Lewis groups of seventy-nine English families and their* 177 *children. The expected and observed absolute numbers of children from the three types of Lewis matings are shown; the expectations being based on the theory of Andresen*

After Race & Sanger (1950).

Mating		Children				
Type	Number	Number observed	*Le*(*a*+)		*Le*(*a*−)	
			Exp.	Obs.	Exp.	Obs.
Le(*a*+) × *Le*(*a*+)	5	13	13·00	13	0·00	0
Le(*a*+) × *Le*(*a*−)	35	80	25·57	30	54·43	50
Le(*a*−) × *Le*(*a*−)	39	84	8·58	9	75·42	75

exception to this theory is the observation of Andresen (1947) that in infants under seven months old some 75% give positive reactions. It appears that, in infancy, the Lewis antigen can be detected on the red cells of individuals who are either heterozygous or homozygous for the gene concerned but in adults only the homozygotes can be detected.

The particular antigenic substance concerned in these reactions is now designated Le^a. It has been found that all individuals whose red cells are Lewis positive also secrete Le^a substance in their saliva, as evidenced by the capacity of their salivas to inhibit anti-Le^a sera (Grubb & Morgan, 1949). It was also discovered that, at any rate in adults, a large proportion of individuals whose red cells fail to react with the serum nevertheless possess Le^a substance in their saliva. The interpretation seems to be that, in adult life, although Le^a substance is not present in detectable amounts in the red blood cells of individuals heterozygous for this gene, nevertheless these people produce the substance in their secretions.

A further serum (anti-Le^b), discovered by Andresen (1948), was found to bear an almost completely antithetical relationship in its reactions to the anti-Le^a serum (Table 20). It is possible that the antigen Le^b defined by its reaction with this serum is determined by a gene allelic to Le^a, but the problem has not been fully resolved. Le^b substance has been identified in the saliva of $Le(a-b+)$ individuals but not in the saliva of individuals $Le(a+b-)$, i.e. individuals homozygous for the gene determining Le^a.

TABLE 20. *Results of testing* 238 *group O bloods with anti-*Le^a *and anti-*Le^b

After Andresen (1948).

Anti-Le^a	Anti-Le^b	Number	Percentage
+	+	0	0·0
+	−	46	19·3
−	+	178	74·8
−	−	14	5·9

THE RELATIONSHIP BETWEEN LEWIS AND SECRETOR

Grubb (1948) made the surprising observation that there is a very close correlation between the possession by an individual of Le^a substance on the red cells and his status with respect to the secretion or non-secretion of the *ABH* substances. Table 21 represents the combined results of Grubb & Morgan (1949) and of Race & Sanger (1950) on this point. All $Le(a+)$ individuals were non-secretors of *ABH*, occasional non-secretors however were $Le(a-)$. The close correlation might at first sight suggest that the Lewis series of allelic genes and the genes determining *ABH* secretion are in fact identical. The occasional exceptions make this hypothesis in its simplest form unacceptable and it is likely that the relationship, though close, is somewhat more complex.

It is evident that the occurrence of these specific blood-group substances in the saliva and the other body fluids is a widespread phenomenon and occurs in most and perhaps all

TABLE 21

Cells	Secretor *ABH*	Non-secretor *ABH*
$Le(a+)$	0	105
$Le(a-)$	289	3

individuals. Frequently more than one such specific substance will be found. Grubb (1951) has characterized 1000 individuals with respect to the presence of *ABH*, Le^a and Le^b substances in their saliva, and his findings are given in Table 22. Three out of the eight possible combinations were not encountered and one was very rare. It is probable that similar specific substances will eventually be found to be present in the 1·3 % of individuals who do not secrete either *ABH*, Le^a or Le^b.

TABLE 22. *Occurrence of different combinations of ABH, Le^a and Le^b substances in salivas of* 1000 *individuals*

After Grubb (1951).

ABH	Le^a	Le^b	Frequency (%)
+	+	+	71·5
+	+	−	0·0
+	−	+	8·3
+	−	−	0·2
−	+	+	0·0
−	+	−	18·7
−	−	+	0·0
−	−	−	1·3

A, *H* AND Le^a SUBSTANCES

The earliest efforts to isolate and identify the substances responsible for the specific blood-group characters were made by extracting erythrocytes with alcohol. The group materials were not homogeneous, but the results demonstrated that the substances were most probably of carbohydrate nature. The materials appeared to be bound up in some way with the lipid constituents of the red cell surface and were water insoluble. The amount present is extremely small, and it was difficult by using such methods to obtain sufficient material for detailed analyses. As a result the methods for the isolation of the specific substances from human red cells are largely undeveloped, and very little is known concerning their exact chemical nature.

The discovery of the water soluble form of these substances in the tissue fluids and secretions opened the way to their isolation in reasonable amounts. Various materials have been used as a starting-point for such investigations, notably, in man, saliva, urine and gastric juice, and, in animal material, pig gastric mucin and commercial peptone. From these animal materials substances with serological specificity corresponding to human group *A* and *H* antigens have been obtained (Meyer, Smyth & Palmer, 1937; Goebel, 1938; Morgan & King, 1943; Bendick, Kabat & Bezer, 1946).

The most potent sources from normal human material were gastric juice and saliva, and even the latter secretion contains less than 100 mg. of group substance per litre (Kabat, Bendick, Bezer & Beiser, 1947). This small yield prompted Morgan & van Heyningen (1944) to examine the fluids of pseudomucinous ovarian cysts for their content of blood-group substances. It was found that these pathological growths, which are not infrequent, may contain quite large amounts of the blood-group substances in their water soluble form. The volume of individual cyst fluids may vary from a few hundred millilitres to several litres and single cysts are sometimes found to contain several grammes of the specific substance. From such starting materials substantially

pure preparations have been obtained of group substances *A* (from cysts of A_1 secretors), Le^a (from *Le*(*a*+) non-secretors *ABH*), and *H* (from *O* secretors) (Morgan & Waddell, 1945; Aminoff, Morgan & Watkins, 1950; Annison & Morgan, 1952). The inhibition end-points found when the substances were titrated against anti-*A*, anti-*H*, and anti-Le^a sera showed that the amount of heterologous specificity in each material was small, certainly less than 1 % of the activity shown with the homologous reagent (Table 23) (Morgan, 1950).

Chemical analysis indicated that the substances could be regarded as complex mucoids made up of a polysaccharide component firmly linked to an amino-acid containing residue. The three substances revealed close similarities in their compositions (Table 24).

TABLE 23. *Serological specificity of isolated human blood-group substances. End-titre of inhibition against two to three agglutinating doses of human serum*

After Morgan (1950).

	Anti-*A*	Anti-*H*	Anti-Le^a
A-substance	1×10^6	$< 1 \times 10^3$	$< 1 \times 10^4$
H-substance	$< 2 \times 10^2$	5×10^5	2×10^2
Le^a-substance	$< 2 \times 10^2$	$< 2 \times 10^2$	1×10^6

TABLE 24. *Analytical figures (average values) for preparations of the human blood-group substances*

After Morgan (1950).

	Rotation (°)	Nitrogen (%)	Acetyl (%)	Hexosamine* (%)	Reduction† (%)	Fucose (%)
A-substance	+15	5·7	9·0	37	56	18
H-substance	−30	5·3	8·6	31	54	14
Le^a-substance	−40	5·0	9·9	32	57	13

* As glucosamine base.
† In terms of glucose equivalent.

The amino-acid residue consists of at least eleven amino-acids, the aromatic and sulphur containing amino-acids being absent or present only in extremely small amounts, while among the others threonine was present in particularly high concentrations—much greater than found in most proteins. The polysaccharide component contains D-galactose, the methyl pentose, L-fucose, and two hexosamines D-glucosamine and D-chondrosamine. The three substances give qualitatively similar amino-acid and sugar chromatograms. Quantitatively there appears to be some difference in the relative amounts of the sugars present. Thus *A* substance contains about 18 % of fucose, three-quarters of which is liberated as free sugar after hydrolysis with acetic acid at 100°, whereas Le^a substance contains only some 13 % fucose, almost the whole of which is split off under the same conditions of hydrolysis. The ratio of D-glucosamine to D-chondrosamine in the *A* substance and *H* substance is very close to one, whereas these two amino-sugars are found together in the Le^a substance in the ratio of about 3 : 1.

The substances after treatment with alkali under rather special conditions yield, on the addition of Ehrlich's reagent, a bright purple red colour. This property has not been encountered

during the examination of many other complex mucopolysaccharides, and Morgan believes that it can be considered a reasonably specific test for the blood-group mucoids or materials closely related to them. He reports (1950) that a mucoid material, of unidentified blood-group character, was isolated from the secretions of an individual belonging to the rare type $Le(a-b-)$ non-secretor ABH, that is to say having none of the recognized group-specific substances in his secretions. This material likewise gave the characteristic and apparently specific colour reaction with Ehrlich's reagent, and presumably indicates the presence of an as yet unrecognized specific substance.

The sedimentation and diffusion constants of the A, Le^a and H substances indicate that the molecular weights are of the order of 250,000.

CONCLUSIONS

It is apparent that the classical blood groups of Landsteiner can no longer be thought of simply in terms of the red blood cells. The genes involved obviously influence the specificities of mucoid materials in all the organs and body fluids. Furthermore, it is probable that all individuals possess one or more such specific mucoids in the secretions. Presumably they must play some significant physiological role, but as yet there is no indication of what this might be.

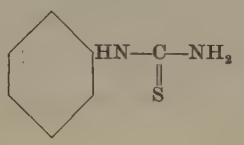
The presence of a particular specific substance in the secretions of an individual may be directly dependent on the presence of genes at more than one locus. Thus an individual secreting substance B must be homozygous or heterozygous for Bernstein's gene **B**, and also not homozygous for **Le**^{a} or non-secretor. Furthermore, the genetic constitution of an individual may lead to the occurrence of particular group-specific substances on the red cells but not in the secretions, and vice versa. Thus an individual homozygous for **B** and **Le**^{a} will have B and Le^a substances in his red cells but only Le^a in his secretions. On the other hand, adults heterozygous for **Le**^{a} may have Le^a substance in their secretions but no detectable amount on their red cells.

The finding that Le^a and A substances have such a close chemical relation to one another is a result of some significance, since their specificities are determined by genes at quite different loci. A picture is emerging of what would appear to be a common or basic molecular framework which develops minor quantitative and perhaps qualitative or stereochemical modification according to the specific action of the gene concerned with its production (Morgan, 1950).

The close similarity of H substance to the others is very interesting. What its precise role really is and whether it can be regarded, as has been postulated on serological grounds (Morgan & Watkins, 1948), as some sort of basic material from which the other specific substances are derived will no doubt emerge in the course of the next few years.

VIII. DIFFERENCES IN TASTE SENSITIVITY TO THE THIOUREAS

Fox (1932) made the curious discovery that people differ very markedly in their ability to taste phenylthiourea



To some individuals this substance is extremely bitter, to others it is virtually tasteless. Approximately 70 % of European populations fall into the former class, the 'tasters', and 30 % into the other class, the so-called 'non-tasters'. At first the differentiation was made by putting

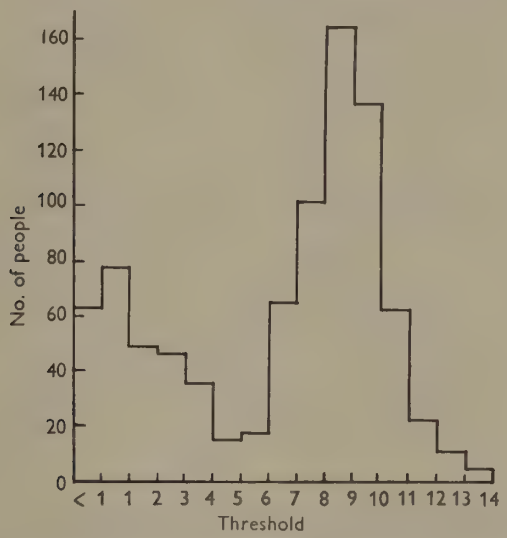


Fig. 27. Taste thresholds for phenylthiourea in 855 people. Threshold concentrations: solution no. 1 = 1·3 g. phenylthiourea per litre; solution no. 2 = half the concentration of no. 1, i.e. 0·65 g. per litre; solution no. 3 = half the concentration of no. 2, i.e. 0·325 g. per litre; and so on. (Based on data of Harris & Kalmus, 1949*a*, and Mohr, 1951.)

a crystal of the substance on a person's tongue or giving them a fairly strong solution of it to taste. Most people could be readily categorized in this way, but there remained a small proportion of people who gave equivocal responses. This was explained by the demonstration of Blakeslee (1932) and subsequent workers (Hartmann, 1939; Falconer, 1947; Harris & Kalmus, 1949*a*), that the distribution of taste thresholds in a population was continuous but bimodal (Fig. 27). That is to say, all grades of sensitivity to this substance occurred but, in general, people tend to clump together at one or other end of the distribution, the 'tasters' being relatively sensitive, the 'non-tasters' relatively insensitive, to the substance. It appears to represent, therefore, a true example of human dimorphism, but the variation in each group is quite extensive and leads to some overlap between the groups. Thus it is not possible to

classify a small proportion of individuals (less than 5 %) confidently in either of the two classes 'tasters' or 'non-tasters'.

Usually in such experiments a series of solutions of increasing dilution are made up and the subject asked to find the weakest solution at which the taste may be detected. A more objective procedure was devised by Harris & Kalmus (1949*a*), based on the ability of individuals to discriminate various solutions of the substance from water. Starting from the higher dilutions and working down, the subject tastes a few millilitres in a tumbler till he first says that he perceives a definite taste. This gives an approximate determination of the threshold. He is now presented with eight tumblers, four of which contain water and four of which contain the solution of concentration determined by the preliminary test. The tumblers are arranged at

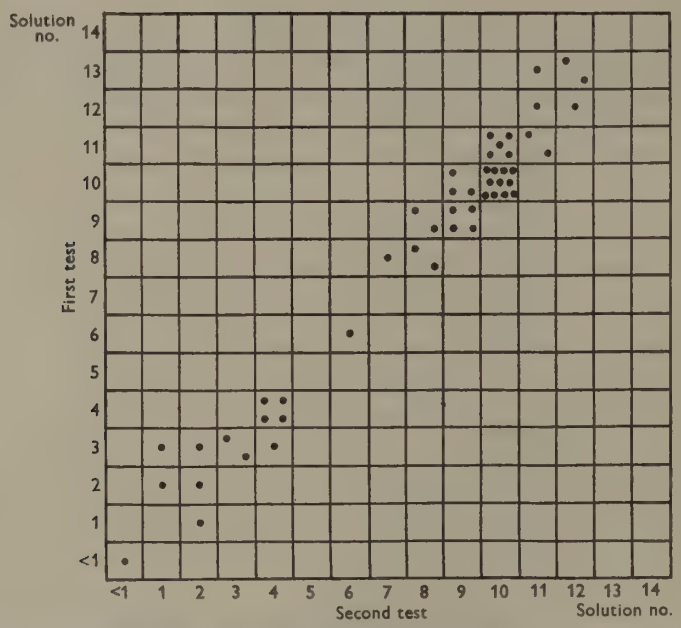


Fig. 28. Results of retesting forty-nine people with the threshold method for phenylthiourea. Solution numbers as in Fig. 27. (After Harris & Kalmus, 1949*a*.)

random and the subject has to separate them into two groups of four. If the two groups are correctly separated the test is repeated with the next higher dilution, and so on, until the subject can no longer discriminate accurately. If, on the other hand, the subject is unable to separate the two groups accurately the test is repeated in the same manner with increasing concentrations till a solution is reached where a completely correct answer is obtained. Serial dilutions, each half as concentrated as the previous one, are quite suitable for most purposes, but of course the method may be elaborated or simplified according to the particular problem in hand. Repeated tests at intervals on the same individuals reveal that there is little variation from time to time and, in fact, any particular person appears to occupy a fairly characteristic position in the general distribution (Fig. 28).

As people get older their overall taste sensitivity appears to decrease, so that the threshold distribution obtained by the above method is shifted in the direction of the higher concentrations (Fig. 29). Both the tasters and the non-tasters are affected in this way, and the relative pro-

portions of the two classes does not appreciably change, though the two modes and also the trough of the distributions all occur at higher concentrations. Females are probably slightly more sensitive, both as tasters and non-tasters, than are males.

Snyder (1932) and Blakeslee (1932), soon after the discovery of this taste polymorphism, studied quite a large number of families. They came to the conclusion that the inability to taste these substances is inherited as a simple Mendelian recessive character. It is doubtful, however, whether this simple hypothesis can adequately explain all the familial data (Boyd, 1950; Harris & Kalmus, 1951), so that, although there seems little doubt that the dimorphism is largely genetically determined, the detailed character of the hereditary processes involved still remains somewhat obscure.

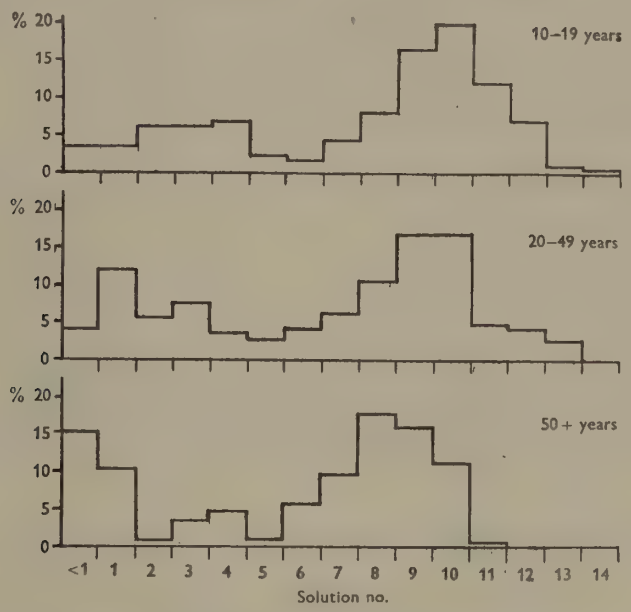
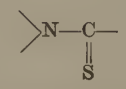


Fig. 29. Threshold distribution for phenylthiourea at different ages. Solution numbers as in Fig. 27.

THE CHEMICAL SPECIFICITY OF THE TASTE RESPONSES

The chemical specificity concerned in these reactions is of considerable interest. Tasters and non-tasters for phenylthiourea appear to taste most other bitter substances, such as quinine or brucine, equally well, and similarly there seems to be no differential response to substances which have sweet or sour tastes. On the other hand, 'tasters' and 'non-tasters' for phenylthiourea have been found to react differently to many compounds which are closely related chemically to this substance. A convenient method of investigating this problem is to determine the taste thresholds by the method described above for phenylthiourea and also for some related substance in a series of subjects. In general it is found that there is either a high correlation between the two sets of values or virtually no relationship at all (Table 25). Thus, thiourea, diphenylthiourea, thioglyoxaline, thiouracil, thio-methylhydantoin give the same dual taste response as phenylthiourea. On the other hand, other closely related compounds, such as urea, diphenylurea, and uracil fail to give this result. A list of some of the substances tested is given

in Table 26, and some of their structures in Fig. 30. It appears that the characteristic grouping common to all the substances behaving in this way is



and presumably this confers the peculiar specificity. One exception to this general rule has been found, namely *sym.* di-*o*-tolyl-thiourea.

TABLE 25

Distribution of taste thresholds of thirty-seven individuals for thiourea and phenylthiourea

Thiourea, solution no.	Phenylthiourea, solution no.													
	<1	1	2	3	4	5	6	7	8	9	10	11	12	13
8	.	.	.	.	.	.	.	.	1	.	.	4	1	.
7	.	.	.	.	.	.	.	.	.	.	2	1	1	1
6	.	.	.	.	.	.	.	.	2	3	5	.	.	.
5	.	.	.	.	.	.	.	.	1	2	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	1	.	.	.
3	1	2	.	.	2	.	1	.	.	.	.	.	.	.
2	1	.	.	1	1	.	.	.	.	.	.	.	.	.
1	.	1	1	1	.	.	.	.	.	.	.	.	.	.

The solution numbers of thiourea represent serial dilutions by 1/2 of a solution containing 1 g./100 ml. Solution 1 is 1 g./100 ml.; solution 2, 0·5 g./100 ml.; solution 3, 0·25 g./100 ml., etc.

The solution numbers of phenylthiourea represent serial dilutions by 1/2 of a solution containing 0·13 g./100 ml. Solution 1 is 0·13 g./100 ml.; solution 2, 0·065 g./100 ml., etc.

Distribution of taste thresholds of thirty-eight individuals for quinine and phenylthiourea

Quinine, solution no.	Phenylthiourea, solution no.													
	<1	1	2	3	4	5	6	7	8	9	10	11	12	13
18	.	.	.	.	1	.	.	.	.	.	.	.	.	.
17	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	1	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.	1	.	.
14	.	.	.	1	.	.	.	.	.	.	.	1	.	.
13	.	.	1	.	.	.	.	.	.	1	.	1	1	.
12	.	.	.	.	.	.	.	.	1	.	3	.	.	1
11	.	2	.	.	2	.	.	.	.	1	1	1	.	.
10	.	.	.	.	.	.	.	.	2	1	1	.	.	.
9	.	.	.	1	.	.	.	1	.	.	.	1	.	.
8	.	.	.	.	.	.	1	.	1	.	1	.	.	.
7	.	1	.	.	.	.	.	.	.	1	1	.	.	.
6	.	.	.	.	.	.	.	.	.	.	1	1	.	.
5	.	.	.	.	1	.	.	1	.	.	.	.	.	.

The solution numbers of quinine represent serial dilutions by 3/4 of a solution containing 0·0187 g./100 ml. (solution 1).

The solution numbers of phenylthiourea are as above.

BIOLOGICAL SIGNIFICANCE

This curious division of human beings into two groups according to how they taste phenylthiourea and related compounds, presumably reflects some quite specific difference in their biochemical make up. It is possible that this difference in taste sensitivity may indicate a much

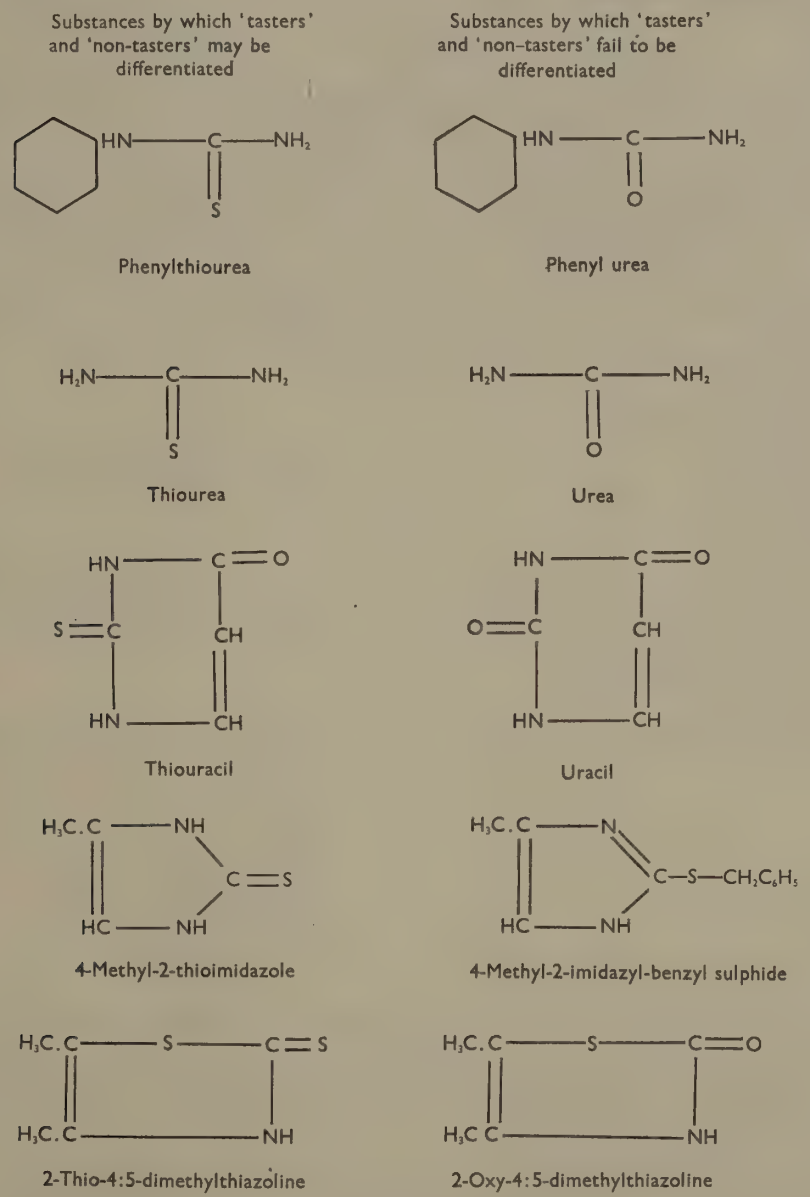


Fig. 30

more generalized difference in the reaction of the two classes of individuals to this series of substances. The question arises as to whether people do in fact come in contact with such substances under ordinary circumstances, and, if so, whether 'tasters' and 'non-tasters' react physiologically to them in different ways.

The natural occurrence of at least one of these compounds in certain foodstuffs nas now been definitely established. Astwood, Greer & Ettlinger (1949) isolated from turnips the substance L-5-vinyl-2-thio-oxazolidone and its presence has also been demonstrated in the seeds of cabbage, rape, kale and brussels sprouts. A related compound, 5:5-dimethyl-2-thio-oxazolidone, had been previously isolated from the seeds of Conringia or hare's ear mustard, a plant of another genus of the Cruciferae (Hopkins, 1938).

TABLE 26

After Fox (1932), Blakeslee & Salmon (1935), Hopkins (1942), Harris & Kalmus (1949*b*), Barnicot, Harris & Kalmus (1951).

Some substances to which 'tasters' and 'non-tasters' for phenylthiourea react differently	Some substances to which 'tasters' and 'non-tasters' for phenylthiourea do not react differently
Thiourea	Urea
Diphenylthiourea	Diphenylurea
Allylthiourea	Phenylurea
Tetra methyl thiourea	
Acetyl thiourea	
p-Chlorophenyl thiourea	
1:3-Diethyl thiourea	
o-Tolylthiourea	*Sym.* di-*o*-tolylthiourea
Thiouracil	Uracil
Methyl thiouracil	
Propyl thiouracil	
Sod. ethyl-(1-methyl-butyl)-thiobarbiturate	Sod. ethyl-(1-methyl-butyl)-barbiturate
2-Thio-4:5-dimethylthiazoline	2-Oxy-4:5-dimethylthiazoline
5:5-Dimethyl-2-thio-oxazoline	
L-5-Vinyl-2-thio oxazolidine	
Thioacetamide	
4-Methyl-2-thioimidazole	4-Methyl-2-imidazyl benzyl sulphide
	Saccharin
	Brucine
	Quinine
	Sucrose
	Acetylsalicylic acid
	Sodium chloride
	Picric acid
	Diphenylguanidine

The best known physiological activity of this group of compounds is their effect on thyroid function. They all, to varying degrees, inhibit the synthesis of thyroxine, and this eventually leads to a compensatory hyperplasia of the thyroid gland consequent to the induced hypothyroidism. As a consequence, substances of this type, for example methyl-thiouracil, are widely used in the treatment of thyrotoxicosis. How far the presence of such goitrogenic substances in vegetables plays any part in the causation of goitre is still not clear, though it is possible that, at least in certain circumstances, they may be of some importance (Bastenie, 1947).

No direct evidence has been obtained which would indicate that, as far as the physiological activities of these substances are concerned, tasters and non-tasters react differently. In view of the possibility that naturally occurring substances of this class might, as a result of their presence in common foodstuffs, play a part in the occurrence of goitre, and that possibly tasters

TABLE 27. *Proportion of 'non-tasters' in patients with goitre*

Group	Total	Percentage non-tasters
Normal subjects	541	31·2
Toxic diffuse goitre (primary thyrotoxicosis)	218	30·1
Nodular goitre	134	41·0
Total	893	32·6

and non-tasters might react differently to them, Harris, Kalmus & Trotter (1949) tested a large number of subjects with goitre for their taste reactions to these compounds. The results are shown in Table 27. The patients were divided into two groups, those with toxic diffuse goitre (primary thyrotoxicosis) and those with nodular goitre. The group with nodular goitre had a higher incidence of non-tasters than the others, and although this suggested that non-tasters might be slightly more susceptible to the development of nodular goitre than tasters, the level of significance in the differences observed was hardly adequate for any definite conclusions to be drawn.

IX. VARIATION IN THE MANIFESTATION OF HEREDITARY CHARACTERS

PHENYLKETONURIA

A genetically determined individual biochemical difference may be detectable in several ways. For example, individuals who are homozygous for the gene causing phenylketonuria show the excretion in abnormal amounts of the substances phenylalanine, phenylpyruvic acid and phenyllactic acid in their urine, their blood phenylalanine level is abnormally raised, they are intellectually retarded, they are slightly stunted in growth, the skull measurements are somewhat smaller than normal, and they show some dilution in the degree of pigmentation of their hair and skin. It seems a reasonable assumption that all these different manifestations may be ultimately traced back to the primary disturbance in metabolism which is believed to be the failure of such individuals to oxidize phenylalanine to tyrosine. However, the details of the various intermediary steps are not known.

If we consider any one of the diverse features of this condition it is evident that, within any group of phenylketonuric individuals, there is considerable variation in the degree of its manifestation. The most notable example is the variation in intellectual retardation. Generally the degree of mental backwardness is severe, and the patients may be classified as idiots or imbeciles, occasionally, however, high-grade feeble-minded individuals are found with apparently the same metabolic disorder.

Each of the different characteristics of this disorder is more or less susceptible to measurement. Thus the biochemical features can be measured by either straightforward chemical techniques or microbiologically; the intellectual retardation can be measured by using the various methods of intelligence testing; and the degree of dilution of hair pigment may be measured by techniques involving the estimation of the amount of light reflected from a sample of the hair at different wavelengths (Fig. 31). When this is done it is possible to construct distributions illustrating the character of the variation within this class of individuals and allowing us to compare it objectively with that found in normals. Such a series of distributions is shown in Fig. 32 and Table 28. Several interesting points emerge. In the first place it is seen that only the biochemical criterion furnishes absolute discrimination between the two classes, the normals and the phenylketonurics. The two distributions of intelligence quotients overlap slightly so that the most high-grade phenylketonurics resemble the individuals in the tail of the distribution of normal individuals. With respect to head size and hair pigmentation, while there is no doubt that, on the average, the two populations differ significantly from one another, nevertheless there is considerable overlapping, and such measurements would form a very unsatisfactory way of differentiating the abnormal type.

Another feature of interest is the extent of the variation, particularly with regard to intellectual ability. The difference between a high-grade feeble-minded phenylketonuric at one end of the distribution and an idiot at the other could reasonably be likened to the difference between extremely intelligent and relatively backward individuals at the two extremes of the distribution of normals.

Such variation can be analysed into two components: the variance within individuals and the

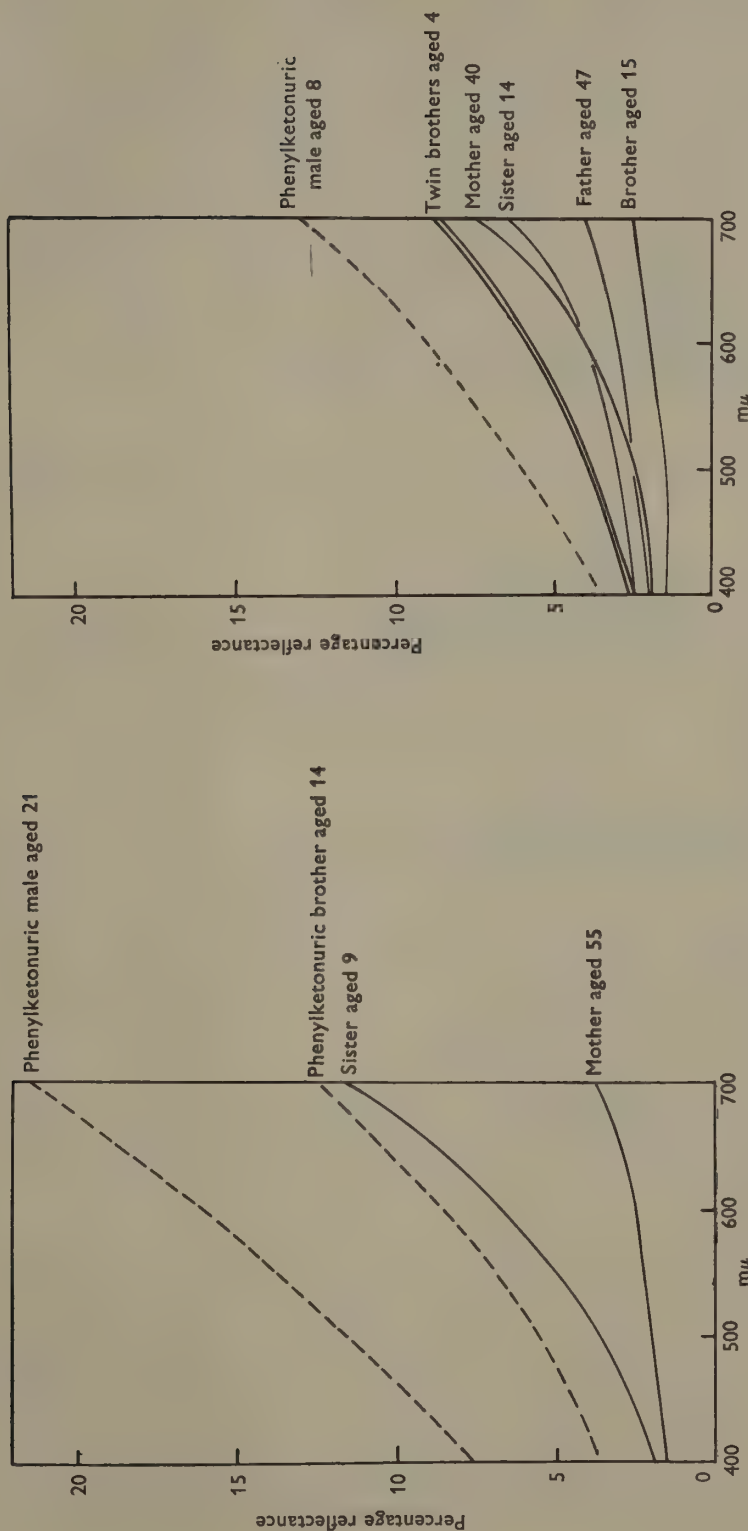


Fig. 31. Percentage reflectance of light at different wave-lengths from samples of hair of individuals in two families each segregating for phenylketonuria. (After Cowie & Penrose, 1951.)

variance between individuals. The former component represents that variation which will be observed when the measurements are repeated on different occasions on the same individual. It reflects both the day-to-day variation in the character as, for example, the variation in phenylalanine excretion on different diets, and also the variation due to errors in measurement, which may be quite large when the technique of estimation happens to be intrinsically difficult. Both these can be regarded in a general sense as environmental sources of variation. The component representing the variation between individuals may be accounted for by either genetical or environmental factors, or more generally by a complex interaction of both. The relative importance of these various components will vary with the particular character trait under consideration. The elucidation of such sources of variation, and of the particular causative factors involved, is in practice extremely difficult. It is of obvious importance in any attempt to understand how a specific inborn biochemical peculiarity may give rise to diverse chemical and morphological results.

TABLE 28. *Means and standard deviations of certain measurements in phenylketonurics and normals*

After Penrose (1951).

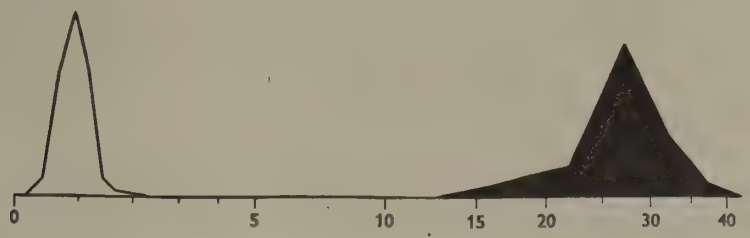
Character	Unit	No. of subjects		Mean measurements ± standard deviations	
		Non-*Phk*	*Phk*	Non-phenylketonuric	Phenylketonuric
Phenylalanine in C.S.F.(1)	mg. %	++	9	0·24 ± 0·06	7·26 ± 0·82
Phenylalanine in plasma(1)	mg. %	17	18	0·95 ± 0·24	27·78 ± 3·90
Phenylpyruvic acid in urine(2)	mg./10 g. N	++	20	(0·00 ± 0·00)	142·35 ± 22·16
Phenyllactic acid in urine(2)	mg./10 g. N	++	20	(0·00 ± 0·00)	63·45 ± 15·62
Phenylalanine in urine (2)	mg./10 g. N	++	20	(0·00 ± 0·00)	29·65 ± 8·68
Intelligence(3)	I.Q.	++	45	(100·00 ± 15·00)	18·9 ± 12·8
Intelligence(4)	I.Q.	++	80	(100·00 ± 15·00)	23·0 ± 14·0
Head size ($l+b$),* males(5)	mm.	70	5	347·8 ± 10·0	324·4 ± 6·7
Head size ($l+b$),* females(5)	mm.	79	9	332·2 ± 10·7	318·6 ± 8·9
Hair colour of sibs (at 500 mμ)(6)	Reflectance %	10	6	3·65 ± 1·32	6·25 ± 2·70
Hair colour of sibs (at 700 mμ)(6)	Reflectance %	10	6	9·05 ± 3·94	13·58 ± 4·78
Hair colour of population corrected for age (at 700 mμ)(6)	Reflectance %	37	22	9·15 ± 5·31	12·95 ± 6·02

++ indicates an indefinite large number. * ($l+b$) indicates length plus breadth in adults.

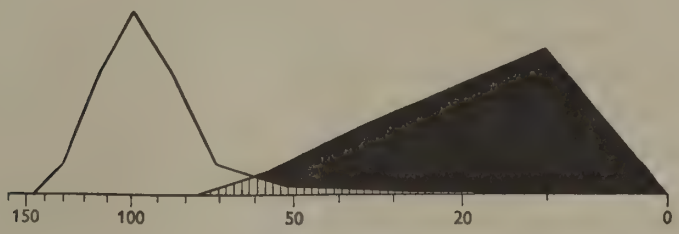
References: (1) Borek *et al.* (1950). (3) Jervis (1937). (5) Penrose (personal observation).
(2) Jervis (1950). (4) Munro (1947). (6) Cowie & Penrose (1951).

The occurrence of a genetical component in such variation is in no way surprising. The group studied is specified by the possession of a mutant gene or genes at a particular locus. They will no doubt differ among themselves in respect to the rest of their genetical make up. Such differences in so far as they influence the manner in which a particular character is expressed can often be demonstrated by measuring the correlation coefficient between affected sibs, or by analysing the variance within and between groups of affected individuals in different sibships or families. Neel and his colleagues (1951), for example, using this approach, were able to show that the variations in the proportion of sickle cell to normal haemoglobin in the heterozygotes for sickle-cell anaemia were largely genetically determined. In the case of phenylketonuria it

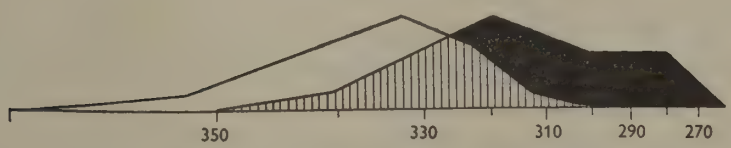
is probable that, at any rate with regard to the degree of intellectual retardation and the physical measurements, the genetical component is quite important. It may be anticipated that ultimately it will be possible to specify more precisely these individual 'modifying' genes which influence the variation in expression of the particular 'main' gene under consideration, and to understand how the chain of processes they set up interact in such a way as to produce the observed variation.



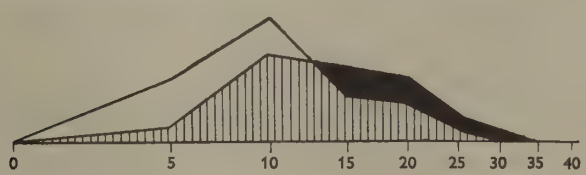
Phenylalanine in blood plasma (mg. %)



Intelligence (Binet, I.Q.)



Head size. Length + breadth in mm. (corrected for sex)



Hair colour. Reflectance % at 700 mμ (corrected for age)

Fig. 32. Frequency distributions: phenylketonurics (right) compared with control populations (left).

CYSTINURIA

The analysis of the causes of the variation within a class of individuals characterized by the recurrence of a specific inborn biochemical difference becomes of some practical importance when this peculiarity leads in some instances but not in others to pathological effects. Thus, in cystinuria where there is an abnormally large excretion of cystine, lysine and arginine, some individuals but not others develop renal calculi. In the absence of renal calculi no ill effects are observed though the abnormal excretion of the cystine, lysine and arginine persists unchanged

throughout life. The repeated formation of renal calculi may have various consequences, as a result of the mechanical effect of their blocking the renal tract. Hydronephrosis, pyelonephritis, and eventually complete loss of kidney function, often ensue. A satisfactory explanation of why only some cystinurics form stones has not yet been forthcoming. Cystine has a much lower solubility than the other amino-acids. Consequently it tends to crystallize out when excreted in large amounts and so the raw material for stone formation is available. The actual concentration of cystine in the urine at any one time is obviously of some importance. This is partly determined by the overall daily excretion of urinary cystine which is known to vary from cystinuric to cystinuric. It is probable that unless a level of about 0·4 g. a day is reached the chances of stone formation are quite remote. On the other hand, many individuals with apparently similar daily outputs differ according to whether or not they form stones. Other factors predisposing to stone formation are, no doubt, the total daily output of fluid which would influence the concentration of cystine at any particular time, and the occurrence of chance renal infections. A more exact knowledge of the factors involved might well lead to the development of therapy completely preventing stone formation in susceptible individuals. In the meanwhile it seems important that the close relatives of all patients with cystine stone formation should have their urine examined quantitatively for cystine. This would enable all predisposed individuals to be identified and kept under observation. Attention to their fluid intake and the early treatment of urinary tract infections no doubt would provide a useful prophylactic approach.

Another example of a condition with a variable clinical manifestation where this kind of investigation may be of direct practical value is the adult type of Fanconi syndrome (Dent & Harris, 1951). The pedigree of one family in which this condition was segregating is shown in Fig. 33. The patient who originally attracted attention was III. 13. She was a housewife aged 41 who had, for the previous seven years, suffered from increasingly severe 'rheumatic' pains in various parts of the body. The pains had gradually restricted her movements and she eventually became completely bedridden. Investigation revealed that she had all the typical features of the adult form of the Fanconi syndrome. X-rays of the skeleton revealed generalized decalcification and multiple 'pseudo-fractures' in the ribs and long bones. Her urine showed gross amino-aciduria, large quantities of serine, glycine, alanine, glutamine, valine, leucine, tyrosine, phenylalanine, lysine, arginine, cystine and proline being present. There was also glycosuria of the renal type, low blood phosphorus (1·5 mg. %), and chronic acidosis (CO_2 combining power 44 vol. %). She was treated with large doses of alkalis, a high calcium intake and massive doses of vitamin D. Within two weeks of beginning the treatment, definite lessening of the bone pains was noted, and X-rays taken a week later showed early healing of the pseudo-fractures. She was later able to walk again with decreasing difficulty and in about four months she was back at home at her housework. After ten months' treatment she was active and well, and the X-rays revealed almost complete healing of the bones. The amino-aciduria, however, remained unchanged. Among her relatives there was no history of anybody else suffering from a similar condition. However, an examination of the urines of some forty of her relatives revealed the fact that two brothers and one sister had biochemically very similar peculiarities although clinically they were quite well. They showed exactly the same type of generalized amino-aciduria, the details of the patterns being remarkably similar in all four individuals. There was also present glycosuria of the renal type, hypophosphataemia, and a mild acidosis. X-rays showed no signs of bony resorption. It seemed probable that sooner or later these sibs would develop

Fig. 33. Pedigree of Fanconi Syndrome. Urines of all individuals were tested except those marked NT. G/A = Generalized amino-aciduria. (After Dent & Harris, 1951.)

the same bony changes as the proposita, but, in view of the favourable results of therapy in her case, it is likely that periodic observation followed by immediate treatment at the first signs of deterioration in their condition should prevent any development of serious disability. The underlying biochemical disturbance in these cases is not entirely understood, but it is possible that the main abnormality is a severe disturbance of renal tubular reabsorption involving many amino-acids, glucose, phosphates and possibly other metabolites (Dent, 1947). The reason why only one out of the four affected individuals should have developed severe bony resorption is still somewhat obscure. The affected sib was younger than two of the others. Apart from the possibility that she may have been predisposed as a result of calcium loss during her two pregnancies, both of which were followed by full periods of lactation, there were no obvious features in her personal history which would account for the strikingly different course of the condition. Possibly further observation of the healthy sibs might suggest the nature of the precipitating factors leading to the bony changes.

GOUT AND HYPERURICAEMIA

Recent studies on gout and hyperuricaemia emphasize the importance of physiological effects arising from differences in sex and age in determining the manifestation of metabolic variation.

Gout is a disorder in which there is an abnormal tendency to precipitate urates in certain tissues, particularly the articular cartilages, synovial membranes and the capsules of joints. It is the inflammatory and degenerative tissue reaction resulting from this that give rise to the clinical arthritis. The clinical course of the disease is extremely variable. Generally there are acute attacks of arthritis at regular intervals, in between which the patient may be quite well and show no disability. Sometimes an individual may have only one attack during his life, but in other cases the attacks recur and eventually a state of chronic gouty arthritis with permanent joint damage and deformity may supervene. The metatarso-phalangeal joints of the big toes are the ones most frequently involved, the next most commonly involved being the small joints of the feet, the ankles, the hands and the wrist. Deposits of urates may also occur in the helix and antihelix of the ear, the olecranon and patellar bursae, and the tendons of the hands and feet. The first attack usually develops in middle life but there is considerable variation in this. Only very rarely does an attack occur in children. In general the earlier the onset of the disorder the more crippling does it tend to be.

Patients with gout consistently show a raised level of blood urate (Table 29). This high level of uric acid in the blood was first observed by A. B. Garrod (1848), and it has been found to be constantly present whether the patient is having gouty attacks or not. It appears to be an essential prerequisite before the arthritis or the gouty deposits can develop. Uric acid is, in man, the end-product of purine metabolism and is derived from the breakdown of nucleoprotein already present in the body tissues or occurring in the food. It is excreted in the urine. In non-gouty individuals an elevation of blood uric acid may occur, for example in leukaemia, in resolving pneumonia, or as a result of an excess purine intake. In such circumstances there is always a great increase in urinary excretion of uric acid. Hyperuricaemia in gout, however, is not accompanied by such an increase in urinary output and, on the average, the daily output of uric acid by the gouty individual is no higher than that in the normal. It appears probable that the intermediary metabolism of the gouty person is normal, and that his hyperuricaemia is accounted for by a limitation of renal excretion of uric acid. This impairment of ability to excrete

uric acid appears to be specific because, at least in the early stages of the condition, there is usually no other demonstrable kidney disease. Marked renal damage, however, may occur in the later stages of the disorder and results in a retention of non-protein nitrogen and a further rise in blood urate.

Although a decreased renal excretion is no doubt responsible for the hyperuricaemia in gout, the mechanism of the formation of urate deposits in the tissues remains uncertain. Uric acid even in much higher concentrations than those found does not readily precipitate from plasma. Thus hyperuricaemia, though an essential feature of gout, is not the sole cause of the urate deposits in the tissues, and some other factors appear necessary for this to occur.

TABLE 29. *Serum urate levels in gouty and normal individuals*

After Jacobsen (1937).

Serum urate level (mg./100 ml.)	Normal individuals		Individuals with gout	
	Males	Females	Males	Females
1– 1·9	.	1	.	.
2– 2·9	8	6	.	.
3– 3·9	13	7	.	.
4– 4·9	23	16	.	.
5– 5·9	16	7	.	.
6– 6·9	3	.	1	.
7– 7·9	.	.	5	.
8– 8·9	.	.	6	.
9– 9·9	.	.	4	.
10–10·9	.	.	1	1
11–11·9	.	.	1	.
12–12·9	.	.	2	.
13–13·9	.	.	.	.
Totals	63	37	20	1

INCIDENCE AND FAMILIAL DISTRIBUTION

Gout is about twenty times as common in men as in women. Its incidence in the general population is by no means rare, though accurate figures are difficult to obtain. It is commonly believed that the disorder was at one time very much more common than it is to-day. Changes in diagnostic standards and the absence of any satisfactory statistical data make it difficult to reach any definite conclusions on this point.

Gout may occur in several members of the same families. This has been known for a long time, and many examples are quoted in the early literature (Scuddamore, 1823; Hutchinson, 1876). There is little doubt that the incidence of the disease among close relatives is elevated, and this has always been assumed to be of genetical significance. There is no increase in parental consanguinity. The cases may be distributed in more than one generation of the same family, and the condition may be apparently transmitted by either sex. The pedigrees have, however, always been found to be somewhat irregular in character and many apparently sporadic cases occur. Such irregularities are not of course entirely surprising in a condition which cannot often be recognized till middle or late life. In such circumstances individuals who may subsequently develop the disorder will, at the time of the investigation, be classified as normal. However,

even allowing for this source of error, the condition has a very sporadic familial distribution. The explanation usually advanced for this kind of 'irregular dominant' familial pattern is that the affected individuals are heterozygous for a gene which predisposes them to the development of the condition. Only a small proportion of the individuals thus predisposed actually develop the clinical symptoms and signs of the disorder. Consequently, while there is a higher incidence of the disease among close relatives of affected individuals than in the general population, the pedigrees necessarily appear irregular. The assumption that the affected individuals are heterozygous rests on the fact that the disorder is relatively frequently identified in more than one generation of the same family. Such a hypothesis naturally raises the question as to whether there is any way of recognizing the clinically symptomless, heterozygous, individuals. Recent work by Talbot (1940), Smyth, Cotterman & Freyberg (1948) and Stecher, Hirsh & Solomons (1949) has thrown new light on this problem.

SYMPTOMLESS HYPERURICAEMIA

These authors have shown quite conclusively that the average level of blood urate among the non-gouty sibs, parents and children of patients with clinical gout is significantly greater than a comparable unrelated series of normal individuals. Among such relatives quite high levels of blood urate may be encountered in the absence of any clinical signs or symptoms of gout, or of any history of such symptoms. Clinically such individuals appear perfectly normal.

Unfortunately, the fasting serum urate level is far from being a fixed individual characteristic. Various environmental factors, in addition to technical errors, are believed to be effective in producing fluctuations in serum urate determinations even when tests are made on individual subjects over short periods of time. Furthermore, because of variations in technique individual determinations are only readily interpreted against norms obtained in the same laboratory. These considerations coupled with the fact that in certain other diseases, the most common being chronic nephritis, the serum urate level may reach high values as a result of the disease process itself, clearly make the interpretation of familial data based on serum urate concentrations a matter of some difficulty. The difficulties are further increased by important sex differences which have been discovered in respect to serum urate concentrations. There is little doubt that the serum urate level in adults is on the average higher among males than among females. The mean reported ratio: $\frac{\text{average female plasma urate}}{\text{average male plasma urate}}$, in ten different investigations, has been 0·84 (Wolfson *et al.* 1949*a*, *b*) (Table 30).

In spite of these difficulties Smyth *et al.* (1948) have been able to bring forward quite strong evidence that, among the immediate relatives of gout patients, the distribution of serum urate level is probably bimodal and that, on this basis, it is possible to classify them into two groups, the 'hyperuricaemics' and the 'normals'. The groups, not surprisingly, overlap to a certain extent (particularly in the females) and consequently some error in classification is inevitable. However, they were able to show that the 'hyperuricaemics', only about 10 % of whom exhibited any clinical signs or symptoms of gout, were distributed in the nineteen families studied much in the way that would be expected if they were heterozygous for an abnormal gene tending to produce the condition. Stecher *et al.* (1949), in a similar investigation, arrived at much the same conclusion.

The conclusion drawn by these authors was that the tendency to high levels of serum urate

is an inherited characteristic. It occurs in individuals heterozygous for a particular mutant gene and it predisposes the individuals concerned to the development of clinical gout. The absence of clinical symptoms in most hyperuricaemics accounted for the apparent irregularity of pedigrees of gout. The great difference between the harmless asymptomatic hyperuricaemia and the severe crippling arthritis of classical gout represents the extreme range of variation in the manifestation of this condition.

TABLE 30. *Ratio of average female to male plasma urate concentrations in different reported studies on normal adults*

After Wolfson *et al.* (1949*a*, *b*).

Author	Males		Females		$\frac{\text{Female}}{\text{Male}}$ ratio
	No.	Av. plasma urate (mg. %)	No.	Av. plasma urate (mg. %)	
Wolfson *et al.* (1949*a*, *b*)	22	5·27	20	4·05	0·77
Jacobsen (1937)	63	4·40	37	4·00	0·91
Mull (1943)	51	4·30	22	3·81	0·89
Mull (1943)	51*	3·23	22	2·30	0·71
Bulger & Johns (1941)	62*	4·40	41	3·40	0·77
Leone (1947)	59*	5·81	49	5·17	0·89
Stecher *et al.* (1949)	296	3·95	294	3·63	0·92
Brøchner-Mortensen (1939)	25	7·62	25	6·35	0·83
Berglund & Frisk (1935)	43	3·20	89	2·70	0·84
Brown (1945)	12	2·84	17	2·39	0·84

* Uricase used.

One of the interesting facts which emerged from these studies was the obvious influence of sex and age on the processes involved. Gout itself occurs twenty times more commonly in men than in women. It is rare in childhood. In the normal the average urate level is higher in males than in females. In males hyperuricaemia does not usually develop before puberty. In the females it does not usually become apparent till even later in life, high values generally being only found after the menopause. Thus Stecher *et al.* (1949) found, among the male relatives of gouty patients, a correlation of serum uric acid with age of $r = +0{\cdot}088 \pm 0{\cdot}129$, but with the female relatives a correlation of $r = +0{\cdot}44 \pm 0{\cdot}09$. It is a tempting hypothesis to suppose that the sex difference in the frequency of clinical gout arises from the fact that a sufficiently high level of serum urate is a necessary prerequisite for this to develop and that this is more often reached in males than in females.

Further evidence that uric acid metabolism is at least partly under the influence of the sex hormones is provided by the work of Wolfson *et al.* (1949*a*, *b*). They have found that there is a marked reduction in the urinary excretion of 17-ketosteroids in patients with gout. It was present in all stages of the disease, even when symptoms were absent. It was not found in patients with other forms of arthritis. The exact significance of this is not yet altogether clear, but Wolfson considers that in the gout patients an abnormal androgen is produced by the adrenal cortex, which, among other things, is concerned in uric acid metabolism, but which when metabolized does not contribute apparently to urinary 17-ketosteroids.

DIFFERENCE IN FREQUENCY IN THE TWO SEXES

Garrod (1923), in his review of the literature of inborn metabolic disorders, came to the conclusion that generally one sex was more frequently affected than the other. Usually males predominated. It is certainly true that, if we examine cases reported in the literature, there is a great preponderance of males over females in conditions such as alkaptonuria, pentosuria, congenital porphyria, haemochromatosis, and, of course, gout. Occasionally, as in acute porphyria, females are in excess. The possibility of sex linkage, that is the determination of the condition by a gene located on the *X*-chromosome, can, in all the above cases, be readily excluded by a consideration of the relative frequencies in the two sexes and by the character of the familial distribution.

If the genetical determinants are autosomal and not sex linked then one would expect the two sexes to be equally affected. How then can the apparent aberrant sex ratios be accounted for? There are, in general, two kinds of explanation which may be advanced. The first hypothesis emphasizes the normal physiological differences between the two sexes. It suggests that, although the primary biochemical disturbance is equally common, the variation in its manifestation is not the same in the two sexes because the series of reactions which determine the manner in which the condition becomes evident are influenced differently by the nature of the physiological background on which they are acting. That this is a reasonable way of looking at the situation in gout, where males are twenty times more frequently affected than females, is suggested by the evidence mentioned above that both in normal and hyperuricaemic individuals the level of blood urate is influenced by physiological factors, probably hormonal, which differ in the two sexes. The same kind of explanation will no doubt eventually be found to account for sex differences in the porphyrias where there seems an obscure and as yet not clearly characterized disturbance in porphyrin metabolism and in haemochromatosis where the metabolism of iron is abnormal.

The second kind of explanation invoked to account for these discrepancies in sex ratio emphasizes sampling peculiarities in the manner in which the data were originally collected and subsequently came to be recorded in the literature. For example, a condition such as pentosuria is perfectly benign and in itself produces no symptoms. It is generally detected only when the urine is examined for sugar, and is consequently not infrequently brought to light as a result of routine urine testing in the course of life-insurance examinations. This tends to favour heavily the identification of the condition in men. Blatherwick, for example, whose cases were mainly collected in this way, found thirty-one male pentosurics and only one female. Lasker *et al.* (1936), studying a series of families, found twenty-three males and seventeen females and, of these, fourteen males and seven females represented the propositi or initial cases which led to the study of any particular family, and nine males and ten females were new cases discovered by testing the urines of their brothers and sisters. Thus it appears probable that a truly random sample of the population would reveal more or less similar frequencies of this condition in the two sexes. Similar considerations apply to renal glycosuria which is identified in much the same way and a preponderance of males is reported in the literature.

In alkaptonuria some twice as many cases have been described in males as in females. The cause of this still remains rather obscure. Hogben *et al.* (1932) collected all the cases which had been reported up till 1931 and divided them into three groups: isolated cases in which no family data were available, cases apparently inherited recessively, and the cases which seemed to fall

TABLE 31. *Sex ratio in published cases of alkaptonuria*

After Hogben *et al.* (1932).

	Males	Females	Total
Isolated cases	28	9	37
Probably recessive (i.e. homozygous) cases			
(*a*) Derived from consanguineous parents	20	7	27
(*b*) Derived from non-consanguineous parents	21	15	36
(*c*) Consanguinity between parents not ascertained	14	6	20
Possibly 'Dominant' (i.e. heterozygous) cases	17	9	26
Total	100	46	146

into the heterozygous class. In all three groups the sex ratio was substantially the same (Table 31). There is no evidence of sex linkage, and the finding of the same abnormal sex ratio, even among cases derived from consanguineous unions, further excluded this possibility. There is no evidence to suggest that physiological differences between the sexes could account for the difference. On such a hypothesis one would have to assume that only approximately half the females genetically predisposed to the development of alkaptonuria actually manifest the condition. The clear-cut character of the disorder and the lack of any suggestion that the metabolism of the aromatic amino-acids is sex-influenced make this unlikely, although at present it cannot be rigorously excluded. If we assume some selection in favour of males in the type of case that comes to be recorded in the literature then we are faced with the problem of how such selection arises. The possibility that the disorder is semi-lethal in the female so that, on the whole, fewer female cases come under observation is extremely unlikely in view of the generally benign clinical nature of the condition. While occasionally cases are detected at life-insurance examinations (Hogben *et al.* 1932) this does not seem to be a sufficiently general cause to account for the discrepancy. The occurrence of ochronosis and arthritis in middle and late life often draws attention to the disability, and certainly is a frequent reason for the particular case to become recorded in the literature. It might be, that if such a development occurred more frequently in men than in women, the preponderance of the male sex in recorded cases could be accounted for.

X. CONCLUDING REMARKS

Although our knowledge of chemical variation in man and of its genetical determination is still very much in its infancy and is confined to a rather haphazard series of examples, new approaches to the subject are being developed, and it is probable that quite rapid advances will occur in the coming years. No doubt such developments will necessitate a considerable reorientation in many of our current viewpoints in this field.

At the moment, the idea that genes in some way control specific steps in biochemical synthesis is perhaps the most useful working hypothesis. It may be supposed that mutations result in the occurrence of genes differing somewhat in properties from the original so that the particular synthetic step concerned is performed either not at all, or at a slower rate, or in some qualitatively different way. Hence the change may result in the complete failure of the synthesis, or the formation of the normal product in limited amounts, or the formation of a product differing in chemical and physical structure from that normally produced. Where we are concerned with the synthesis of complex molecules, such as proteins or polysaccharides, such differences in structure may be extremely subtle; the resulting product, while differing in one or more properties from the substance as usually encountered, may in most other respects resemble it closely. Thus the different genetically determined haemoglobins no doubt resemble normal haemoglobin very closely in most of their characteristics, and differ markedly only in particular properties such as solubility or electrophoretic mobility. Similarly, we may expect that the various antigenically specific mucoids of the *ABO* blood-group system are extremely similar in most of their characteristics, the large differences in serological properties being dependent on very subtle differences in chemical and physical structure. Where a protein functions as an enzyme it may well be that a slight change in structure could lead to a considerable, if not complete, loss in its ability to perform a particular catalysis, and in this way blocks in intermediary metabolism or other enzymatically controlled processes can be envisaged.

Subtle differences in the formation of particular complex molecules may lead to gross physiological and pathological disturbances. They may, on the other hand, have no obvious deleterious consequences, and no doubt all grades of effect on the health and fitness of the individuals concerned can be encountered. Furthermore, individuals with a particular kind of biochemical make-up may be at an advantage in certain kinds of environment and at a disadvantage in others. Thus it is doubtful whether any sharp distinction between 'normal' and 'pathological' variation can be drawn.

It may be expected that research in the next few years will lead not only to the progressive characterization of the known biochemical variants but also to the discovery of many new ones. A large number of diseases and morphological abnormalities which are genetically determined in a more or less simple way are known in man, and we can expect that it will eventually become possible to characterize the underlying disturbance in these conditions in biochemical terms. As happened in the case of sickle-cell disease, unsuspected causes of heterogeneity will no doubt be discovered and the extension of such studies will perhaps lead, as it did in this case, to the uncovering of biochemical variation among apparently normal and healthy individuals. A direct attack on the biochemical variation among randomly selected 'normal' individuals may also

prove profitable, though in practice the development of techniques sufficiently sensitive to cope with subtle individual differences and yet suitable for extensive population surveys and family studies is a formidable difficulty. In the case of rare recessive abnormalities, such as phenylketonuria, alkaptonuria, and the like, it is possible that an intensive study of known heterozygotes, that is the parents and children of such individuals, would lead to the detection of chemical peculiarities resulting from the presence of the abnormal gene in single dose. Such peculiarities, though perhaps slight in comparison with the effects encountered in the homozygotes, would be of considerable importance in attempting to understand the nature of the action of the genes concerned. Individuals heterozygous for such genes are relatively common. For example, some 1 % of the population of the United Kingdom are heterozygous for the gene which, in homozygous form, produces phenylketonuria. Such investigations therefore may afford one way in which the 'normal' biochemical variation within a population may be approached.

REFERENCES

Abderhalden, E. (1903). Familiäre Cystindiathese. *Hoppe-Seyl. Z.* **38**, 557.

Abderhalden, E. & Falta, W. (1903). Die Zusammensitzung der Bluterweisstoffe in einem Falle von Alkaptonurie. *Hoppe-Seyl. Z.* **39**, 143.

Aminoff, D., Morgan, W. T. J. & Watkins, W. M. (1950). Studies on immunochemistry. 8. The isolation and properties of the human blood group A substance. *Biochem. J.* **46**, 426.

Andresen, P. H. (1947). Blood group with characteristic phenotypical aspects. *Acta path. microbiol. scand.* **24**, 616.

Andresen, P. H. (1948). The blood group system L. A new blood group L_2. A case of epistasy within the blood groups. *Acta path. microbiol. scand.* **25**, 728.

Annison, E. F. & Morgan, W. T. J. (1952). Studies in immunochemistry. 10. The isolation and properties of Lewis (Le^a) human blood group substance. *Biochem. J.* **50**, 460.

Astwood, E. B., Greer, M. A. & Ettlinger, M. G. (1949). The antithyroid factor of yellow turnip. *Science*, **109**, 631.

Bachmann, G. & Haldi, J. (1937). Comparative study of R.Q. following ingestion of glucose and fructose as affected by lactic acid and CO_2 changes in blood. *J. Nutrition*, **13**, 157.

Barcroft, H., Gibson, Q. H., Harrison, D. C. & McMurray, J. (1945). Familial idiopathic methaemoglobinaemia and its treatment with ascorbic acid. *Clin. Sci.* **5**, 145.

Barnicot, N. A., Harris, H. & Kalmus, H. (1951). Taste thresholds of further eighteen compounds and their correlation with P.T.C. thresholds. *Ann. Eugen., Lond.*, **16**, 119.

Bastenie, P. A. (1947). Diseases of the thyroid gland in Occupied Belgium. *Lancet*, **1**, 789.

Beet, E. A. (1949). The genetics of sickle-cell trait in a Bantu tribe. *Ann. Eugen., Lond.*, **14**, 279.

Bell, J. (1940). The determination of the consanguinity rate in the general hospital population in England and Wales. *Ann. Eugen., Lond.*, **10**, 370.

Bendick, A., Kabat, E. A. & Bezer, A. E. (1946). Immunochemical studies on blood groups; properties of purified blood group A substances from individual hog stomach linings. *J. Exp. Med.* **83**, 485.

Bensley, E. H., Rea, L. J. & Mills, E. S. (1938). Familial idiopathic methaemoglobinaemia. *Quart. J. Med.*, N.S., **7**, 325.

Berglund, H. & Frisk, A. R. (1935). Uric acid elimination in man. *Acta med. scand.* **86**, 233.

Bernstein, F. (1925). Zusammenfassende Betrachtungen über die erblichen Blutstrukturen des Menschen. *Z. indukt. Abstamm.- u. VererbLehre*, **37**, 237.

Bertinshaw, D., Lawler, S. D., Holt, H. A., Kirman, B. H. & Race, R. R. (1950). The combination of blood groups in a sample of 475 people in a London hospital. *Ann Eugen., Lond.*, **15**, 234.

Beyer, K. H., Wright, L. D., Russo, H. F., Skeggs, H. R. & Shaner, G. A. (1947). The renal clearance of essential amino-acids: their competition for reabsorption by the renal tubules. *Amer. J. Physiol.* **151**, 202.

Blakeslee, A. F. (1932). Genetics of sensory thresholds: taste for phenylthiocarbamide. *Proc. Nat. Acad. Sci., Wash.*, **18**, 120.

Blakeslee, A. F. & Salmon, T. N. (1935). Genetics of sensory thresholds: individual taste reactions for different substances. *Proc. Nat. Acad. Sci., Wash.*, **21**, 84.

Blatherwick, N. R. Quoted by Lasker, Enklewitz & Lasker (1936).

Block, R. J., Jervis, G. A., Bolling, D. & Webb, M. (1940). Chemical and metabolic studies on phenylalanine; amino-acid content of tissue proteins of normal and phenylpyruvic oligophrenic individuals. *J. Biol. Chem.* **134**, 567.

Blum, L. (1908). Über der Abbau aromatischer Substanzen im menschlichen Organismus. *Arch. exp. Path. Pharmak.* **59**, 268.

Borek, E., Brecher, A., Jervis, G. A. & Waelsch, H. (1950). Oligophrenia phenylpyruvica. II. Constance of the metabolic error. *Proc. Soc. Exp. Biol., N.Y.*, **75**, 86.

Bowcock, H. M. (1929). Familial glycosuria. A report of a family with eighteen cases of glycosuria. *Ann. Intern. Med.* **2**, 923.

Boyd, W. C. (1950). *Genetics and the Races of Man.* Oxford: Blackwell.

Bridge, E. M. & Holt, L. E. (1945). Glycogen storage disease; observations on pathologic physiology of two cases of hepatic form of the disease. *J. Pediat.* **27**, 299.

Brøchner-Mortensen, K. (1939). Arthritis urica. *Bibl. Laeger*, **131**, 51.

Brown, H. (1945). The determination of uric acid in human blood. *J. Biol. Chem.* **158**, 601.

Bulger, H. A. & Johns, H. E. (1941). The determination of plasma uric acid. *J Biol. Chem.* **140**, 427.

Burki, E. (1941). Über Augenveränderungen bei der Cystinkrankheit. *Ann. Paediat.* **156**, 324.

Callender, S. T. E., Nickel, J. F., Moore, C. V. & Powell, E. O. (1949). Sickle-cell disease; studied by measuring survival of transfused red blood cells. *J. Lab. Clin. Med.* **34**, 90.

Cori, G. T. & Cori, C. F. (1952). Glucose-*b*-phosphatase of the liver in glycogen storage disease. *J. Biol. Chem.* **199**, 661.

Cowie, V. & Penrose, L. S. (1951). Dilution of hair colour in phenylketonuria. *Ann. Eugen., Lond.*, **15**, 297.

van Creveld, S. (1939). Glycogen disease. *Medicine, Baltimore*, **18**, 1.

van Creveld, S. (1952). Glycogen disease. *Arch. Dis. Childh.* **27**, 113.

Crumpler, H. R., Dent, C. E., Harris, H. & Westall, R. G. (1951). β-Amino*iso*butyric acid (α-methyl-β-alanine): a new amino-acid obtained from human urine. *Nature, Lond.*, **167**, 307.

Dahr, P. (1939). Beitrag zur Verbreitung des anti-O (α_2)-agglutinins. *Klin. Wschr.* **18**, 471.

Dann, M., Marples, E. & Levine, S. Z. (1943). Phenylpyruvic oligophrenia: report of a case in an infant with quantitative chemical studies of the urine. *J. Clin. Invest.* **22**, 87.

Darling, R. C. & Roughton, F. J. W. (1942). The effect of methaemoglobin on the equilibrium between oxygen and haemoglobin. *Amer. J. Physiol.* **137**, 56.

Deeny, J., Mundrik, E. J. & Rogan, T. J. (1943). Familial idiopathic methaemoglobinaemia with a note on the treatment of two cases with ascorbic acid. *Brit. Med. J.* **1**, 721.

Dent, C. E. (1947). The amino-aciduria in Fanconi syndrome. A study making extensive use of techniques based on paper partition chromatography. *Biochem. J.* **41**, 240.

Dent, C. E. (1949). Partition chromatography: application to study of amino-acid and protein metabolism. *Biochem. Soc. Symp.* no. 3. Cambridge University Press.

Dent, C. E. (1950). Personal communication.

Dent, C. E. & Harris, H. (1951). The genetics of 'cystinuria'. *Ann. Eugen., Lond.*, **16**, 60.

Dent, C. E. & Rose, G. A. (1951). Amino-acid metabolism in cystinuria. *Quart. J. Med.*, N.S., **20**, 205.

Deuel, H. J. (1936). Intermediary metabolism of fructose and galactose. *Physiol. Rev.* **16**, 173.

von Dungern, E. & Hirszfeld, L. (1910). Ueber Vererbung gruppenspezifischer Strukturen des Blutes. *Z. ImmunForsch.* **6**, 284.

von Dungern, E. & Hirszfeld, L. (1911). Ueber Gruppenspezifische Strukturen des Blutes III. *Z. ImmunForsch.* **8**, 526.

Eder, H. A., Finch, C. & McKee, R. W. (1949). Congenital methemoglobinemia. A clinical and biochemical study of a case. *J. Clin. Invest.* **28**, 265.

Edhem, Erden, F. & Steinitz, K. (1938). Etudes sur un cas de lévulosurie essentielle. *Acta med. scand.* **97**, 455.

Ellis, R. W. B. & Payne, W. W. (1936). Glycogen disease (von Gierke's disease, hepatonephromegalia glycogenica). *Quart. J. Med.*, N.S., **5**, 31.

Embden, H. (1893). Beitrag zur Kenntniss der Alkaptonurie. 1. Ueber einen neuen Fall von Alkaptonurie. *Z. phys. Chem.* **17**, 182.

Emmel, V. E. (1917). A study of the erythrocytes in a case of severe anaemia with elongated and sickle-shaped red blood cells. *Arch. Intern. Med.* **20**, 586.

Enklewitz, M. & Lasker, M. (1933). Studies in pentosuria. *Amer. J. Med. Sci.* **186**, 539.

Esser, M. (1941). Zur Diagnose der Cystinkrankheit. *Ann. Paediat.* **156**, 344.

Falconer, D. S. (1947). Sensory thresholds for solutions of phenylthiocarbamide. *Ann. Eugen., Lond.*, **13**, 211.

Falta, W. (1904). Der Eiweisstoffwechsel bei der Alkaptonurie. *Dtsch. Arch. klin. Med.* **81**, 231.

Fisher, R. A. (1934). The effect of methods of ascertainment upon the estimation of frequencies. *Ann. Eugen., Lond.*, **6**, 13.

Følling, A. (1934*a*). Über Ausscheidung von Phenylbrenztraubensäure in den Harn also Stoffwechselanomalie in verbindung mit Imbezillität. *Hoppe-Seyl. Z.* **227**, 169.

Følling, A. (1934*b*). Utskillelse av fenylpyroduesyr i urinen vom stofskifteanomali forbindelse med imbecilitet. *Nord. med. Tidskr.* **8**, 1054.

Følling, A., Closs, K. & Gammes, T. (1938). Vorläufige Schlüssfolgerungen aus Belastungsversuchen mit Phenylalanine an Menschen und Tieren. *Hoppe-Seyl. Z.* **256**, 1.

Følling, A., Mohr, O. L. & Ruud, L. (1945). Oligophrenia phenylpyruvica. A recessive syndrome in man. *Norske Videnskaps-Akad. i Oslo Mat.-Naturv. Klasse*, **13**.

Fowler, D., Harris, H. & Warren, F. L. (1952). Plasma cystine levels in cystinuria. *Lancet*, **1**, 544.

Fox, A. L. (1932). The relationship between chemical constitution and taste. *Proc. Nat. Acad. Sci., Wash.*, **18**, 115.

Friedenreich, V. & Hartmann, G. (1938). Über die Verteilung der Gruppen Antigene im Organismus der sogenannten 'ausscheider' und 'nichtausscheider'. *Z. ImmunForsch.* **92**, 141.

FRIEDMAN, M., SELZER, A., SUGANNAN, J. & SOKOLOV, M. (1942). Renal blood flow, glomerular filtration rate and degree of tubular reabsorption of glucose in renal glycosuria. *Amer. J. Med. Sci.* **204**, 22.

FROMHERZ, K. & HERMANNS, L. (1914). Ueber den Abbau der aromatischen Aminosäuren im Tierkörper nach versuchen am Normalen und am Alkaptonuriker. *Hoppe-Seyl. Z.* **91**, 194.

GARROD, A. B. (1848). Observations on certain pathological conditions of the blood and urine, in gout, rheumatism and Bright's disease. *Med.-chir. Trans.* **31**, 83.

GARROD, A. E. (1902). The incidence of alkaptonuria. *Lancet*, **2**, 1616.

GARROD, A. E. (1908). The Croonian Lectures on inborn errors of metabolism. *Lancet*, **2**, 1, 73, 142, 214.

GARROD, A. E. (1923). *Inborn Errors of Metabolism*, 2nd edn. Oxford: University Press.

GIBSON, Q. H. (1943). The reduction of methaemoglobin by ascorbic acid. *Biochem. J.* **37**, 615.

GIBSON, Q. H. (1948). The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia. *Biochem. J.* **42**, 13.

GIBSON, Q. H. & HARRISON, D. C. (1947). Familial idiopathic methaemoglobinaemia. *Lancet*, **2**, 941.

VON GIERKE, E. (1929). Hepato-nephromegalia glykogenica (Glycogenseicherkrankheit der Leber und Nieren). *Beitr. path. Anat.* **82**, 497.

GOEBEL, W. F. (1938). Isolation of blood group A specific substance from commercial peptone. *J. Exp. Med.* **68**, 221.

GRUBB, R. (1948). Correlation between Lewis blood group and secretor character in man. *Nature, Lond.*, **162**, 933.

GRUBB, R. (1951). Observations on the human group system Lewis. *Acta path. microbiol. scand.* **28**, 61.

GRUBB, R. & MORGAN, W. T. J. (1949). The 'Lewis' blood group characters of erythrocytes and body fluids. *Brit. J. Exp. Path.* **30**, 198.

GRUTTERINK, A. & VAN DEN BERGH, H. (1907). Over Alkaptonurie. *Ned. Tijdschr. Geneesk.* **2**, 1117.

GULLIVER, G. (1840). Observations on blood corpuscles of certain species of genus cervus. *Edinb. & Dubl. Philos. Mag. & J. Sci.* **17**, 330.

HAHN, E. V. & GILLESPIE, E. B. (1927). Sickle-cell anaemia; report of a case greatly improved by splenectomy; experimental study of sickle-cell formation. *Arch. Intern. Med.* **39**, 233.

HALDANE, J. B. S. (1938). The estimation of the frequencies of recessive conditions in man. *Ann. Eugen., Lond.*, **8**, 255.

HALDANE, J. B. S. (1950). Biochemical aspects of genetics, 1. *Biochem. Soc. Symp.* no. 4. Cambridge University Press.

HARDY, G. H. (1908). Mendelian proportions in a mixed population. *Science*, **28**, 49.

HARRIS, H. & KALMUS, H. (1949*a*). The measurement of taste sensitivity to phenylthiourea (P.T.C.). *Ann. Eugen., Lond.*, **15**, 24.

HARRIS, H. & KALMUS, H. (1949*b*). Chemical specificity in genetical differences of taste sensitivity. *Ann. Eugen., Lond.*, **15**, 32.

HARRIS, H. & KALMUS, H. (1951). The distribution of taste thresholds for phenylthiourea of 384 sib pairs. *Ann. Eugen., Lond.*, **16**, 226.

HARRIS, H., KALMUS, H. & TROTTER, W. R. (1949). Taste sensitivity to phenylthiourea in goitre and diabetes. *Lancet*, **2**, 1038.

HARRIS, J. W. (1950). Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle-cell haemoglobin solutions. *Proc. Soc. Exp. Biol., N.Y.*, **75**, 197.

HARTMANN, G. (1939). Application of individual taste difference towards phenylthiocarbamide in genetic investigations. *Ann. Eugen., Lond.*, **9**, 123.

HARTMANN, G. (1941). *Group Antigens in Human Organs*. Copenhagen: Munksgaard.

HEERES, P. A. & VOS, H. (1929). Fructosuria. Report of a case. *Arch. Intern. Med.* **44**, 47.

HERRICK, J. B. (1910). Peculiar elongated and sickle-shaped red corpuscles in case of severe anaemia. *Arch. Intern. Med.* **6**, 517.

HIER, S. W. (1947). Influence of ingestion of single amino-acids on the blood level of free amino-acids. *J. Biol. Chem.* **171**, 813.

HIMWICH, H. E. & FAZEKAS, J. F. (1940). Cerebral metabolism in mongolian idiocy and phenylpyruvic oligophrenia. *Arch. Neurol. Psychiat.* **44**, 1213.

HIRSZFELD, L. (1947). The transition forms of blood groups. *J. Immunol.* **55**, 141.

HIRSZFELD, L. & HALBER, W. (1928). Ueber gegenseitige Beziehungen gruppenspezifischer Strukturen bei Menschen und Tieren. *Z. ImmunForsch.* **59**, 17.

HIRSZFELD, L. & HIRSZFELD, H. (1919). Serological differences between the blood groups of different races. *Lancet*, **2**, 675.

HITZENBERGER, K. (1933). Autoxische Zyanose (Intraglobuläre Methämoglobinämie). *Wien. Arch. inn. Med.* **23**, 85.

HJARNE, U. (1927). A study of orthoglycaemic glycosuria with particular reference to its hereditability. *Acta med. scand.* **67**, 422.

HOGBEN, L. T. (1931). The genetic analysis of familial traits. I. Single gene substitutions. *J. Genet.* **25**, 97.

HOGBEN, L. T., WORRALL, R. L. & ZIEVE, I. (1932). The genetic basis of alkaptonuria. *Proc. Roy. Soc. Edinb.* **52**, 264.

HOOKER, S. B. & ANDERSON, L. M. (1921). The specific antigenic properties of the four groups of human erythrocytes. *J. Immunol.* **6**, 419.

HOPKINS, C. Y. (1938). A sulphur-containing substance from the seed of *Conringia orientalis*. *Canad. J. Res.* B, **16**, 341.

HOPKINS, C. Y. (1942). Taste differences in compounds having the NCS linkage. *Canad. J. Res.* B, **20**, 268.

HÖRLEIN, H. & WEBER, G. (1948). Ueber chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobins. *Dtsch. Med. Wschr.* **73**, 476.

HOUSTON, J. C. & MERIVALE, W. H. H. (1949). Renal glycosuria in a family. *Guy's Hosp. Rep.* **98**, 233.

HUTCHINSON, J. (1876). On the hereditary transmission of gout. *Med. Times & Gaz.* **1**, 543.

ITANO, H. A. (1951). A third abnormal haemoglobin associated with hereditary haemolytic anaemia. *Proc. Nat. Acad. Sci., Wash.*, **37**, 775.

ITANO, H. A. & NEEL, J. V. (1950). A new inherited abnormality of human haemoglobin. *Proc. Nat. Acad. Sci., Wash.*, **36**, 613.

JACOBSEN, B. M. (1937). The uric acid in the serum of gouty and non-gouty individuals. Its determination by Folin's recent method and its significance in the diagnosis of gout. *Ann. Intern. Med.* **11**, 1277.

JERVIS, G. A. (1937). Phenylpyruvic oligophrenia. *Arch. Neurol. Psychiat.* **38**, 944.

JERVIS, G. A. (1938). Metabolic investigations on a case of phenylpyruvic oligophrenia. *J. Biol. Chem.* **126**, 305.

JERVIS, G. A. (1939). The genetics of phenylpyruvic oligophrenia. *J. Ment. Sci.* **85**, 719.

JERVIS, G. A. (1947). Studies on phenylpyruvic oligophrenia. The position of the metabolic error. *J. Biol. Chem.* **169**, 651.

JERVIS, G. A. (1950). Excretion of phenylalanine and derivations in phenylpyruvic oligophrenia. *Proc. Soc. Exp. Biol., N.Y.*, **75**, 83.

JERVIS, G. A., BLOCK, R. J., BOLLING, D. & KANZE, E. (1940). Chemical and metabolic studies on phenylalanine; phenylalanine content of blood and spinal fluid in phenylpyruvic oligophrenia. *J. Biol. Chem.* **134**, 105.

JONSSON, B. (1944). Blutgruppenstudien mit Japanischen und Schwedischen aalseren. *Acta path. microbiol. scand.*, Suppl. 54, 456.

KABAT, E. A., BENDICK, A., BEZER, A. E. & BEISER, S. M. (1947). Immunochemical studies on blood groups; preparation of blood group A substances from human sources and comparison of their chemical and immunochemical properties with those of blood group *A*. *J. Exp. Med.* **85**, 685.

KAPLAN, E., ZUELZER, W. W. & NEEL, J. V. (1951). A new inherited abnormality of haemoglobin and its interaction with sickle-cell haemoglobin. *Blood*, **6**, 1240.

KATSCH, G. & METZ, E. (1927). Der Nachweis der Homogentisinsaure im serum des Alkaptonurikers. *Dtsch. Archiv. klin. Med.* **157**, 143.

VON KÖRBER, E. (1866). Ueber Differenzen des Blutfarbstoffes. Inaug. Dissert. Dorpat.

LANDSTEINER, K. (1901). Ueber agglutinationserscheinungen normalen und menschlischen Blutes. *Wien klin. Wschr.* **14**, 1132.

LANDSTEINER, K. & LEVINE, P. (1928). On the inheritance of agglutinogens of human blood demonstrable by immune agglutinins. *J. Exp. Med.* **48**, 731.

LANYAR, F. & LIEB, H. (1931). Die quantitätive Bestimmung der Homogentisinsäure im Blutserum und in der Milch des Alkaptonurikers. *Hoppe-Seyl. Z.* **203**, 135.

LASKER, K., ENKLEWITZ, M. & LASKER, G. W. (1936). The inheritance of L-xyloketosuria. (Essential pentosuria.) *Hum. Biol.* **8**, 243.

LASKER, M. (1941). Essential fructosuria. *Hum. Biol.* **13**, 51.

LAWRENCE, R. D. (1940). Renal threshold for glucose: normal and in diabetics. *Brit. Med. J.* **1**, 766.

LEAF, G. & NEUBERGER, A. (1948). The preparation of homogentisic acid and of 2:5-dihydroxyphenylethylamine. *Biochem. J.* **43**, 606.

LEHRS, H. (1930). Ueber gruppenspezifische eigenschaften des menschlichen Speichels. *Z. ImmunForsch.* **66**, 175.

LENZ, F. (1919). Die Bedeutung der statistisch ermittelten Belastung mit Blutverwandschaft der Eltern. *Münch. med. Wschr.* **66**, 1340.

LEONE, E. (1947). La concentrazione dell' acido urico nel sangue. *Boll. Soc. ital. Biol. sper.* **23**, 1.

LEVINE, S. Z., DANN, M. & MARPLES, E. (1943). A defect in the metabolism of tyrosine and phenylalanine in premature infants. VII. Demonstration of the irreversible conversion of phenylalanine to tyrosine in human beings. *J. Clin. Invest.* **22**, 551.

Lewis, E. O. (1929). Report on an investigation into the incidence of mental defect in six areas, 1925–27. *Report of the Mental Deficiency Committee*, Part IV. London: H.M.S.O.

Lewis, H. B. (1932). The occurrence of cystinuria in healthy young men and women. *Ann. Intern. Med.* **6**, 183.

Lian, C., Frumusan, P. & Sassier, M. (1939). Methemoglobinemie congenitale et familiale. Action favorable de l'acide ascorbique. *Bull. Med. Soc. Med. Hop. Paris*, **55**, 1194.

Liquori, A. M. (1951). Presence of foetal haemoglobin in Cooley's anaemia. *Nature, Lond.*, **167**, 950.

McCune, D. J., Mason, H. H. & Clarke, H. T. (1943). Intractable hypophosphataemic rickets with renal glycosuria and acidosis (Fanconi syndrome). *Amer. J. Dis. Child.* **65**, 81.

Marble, A. (1932). Renal glycosuria. *Amer. J. Med. Sci.* **183**, 811.

Margolis, J. I. (1929). Chronic pentosuria and migraine. *J. Amer. Med. Ass.* **93**, 173.

Mason, H. H. & Turner, M. E. (1935). Chronic galactemia; report of a case with studies on carbohydrates. *Amer. J. Dis. Child.* **50**, 359.

Medes, G. (1932). A new error of tyrosine metabolism: Tyrosinosis. *Biochem. J.* **26**, 917.

Meyer, K., Smyth, E. M. & Palmer, J. W. (1937). On glycoproteins, polysaccharides from pig gastric mucosa. *J. Biol. Chem.* **119**, 73.

Mohr, J. (1951). Taste sensitivity to phenylthiourea in Denmark. *Ann. Eugen., Lond.*, **16**, 282.

Morgan, W. T. J. (1950). Nature and relationships of the specific products of the human blood group and secretor genes. *Nature, Lond.*, **166**, 300.

Morgan, W. T. J. & van Heyningen, R. (1944). Occurrence of *A*, *B* and *O* blood group substances in pseudo-mucinous ovarian cyst fluids. *Brit. J. Exp. Path.* **25**, 5.

Morgan, W. T. J. & King, H. K. (1943). Studies in immunochemistry; isolation from hog gastric mucin of polysaccharide-amino-acid complex possessing blood group *A* specificity. *Biochem. J.* **37**, 640.

Morgan, W. T. J. & Waddell, M. B. R. (1945). A specific blood group *O* substance. *Brit. J. Exp. Path.* **26**, 387.

Morgan, W. T. J. & Watkins, W. M. (1948). The detection of a product of the blood group *O* gene and the relationship of the so-called *O* substance to the agglutinogens *A* and *B*. *Brit. J. Exp. Path.* **29**, 159.

Moss, A. R. & Schoenheimer, R. (1940). The conversion of phenylalanine to tyrosine in normal rats. *J. Biol. Chem.* **135**, 415.

Mourant, A. E. (1946). A 'new' human blood group antigen of frequent occurrence. *Nature, Lond.*, **158**, 237.

Moureau, P. (1946). L'antigène *O* dans ses rapports avec la génétique et la sérologie. *Rev. Hémat.* **1**, 266.

Mull, J. W. (1943). Determination of uric acid in whole blood and serum. *J. Lab. Clin. Med.* **28**, 1038.

Munro, T. A. (1947). Phenylketonuria: data on forty-seven British families. *Ann. Eugen., Lond.*, **14**, 60.

Neel, J. V. (1949). The inheritance of sickle-cell anaemia. *Science*, **110**, 64.

Neel, J. V. (1951). The inheritance of the sickling-phenomenon, with particular reference to sickle-cell disease. *Blood*, **6**, 389.

Neel, J. V., Wells, I. C. & Itano, H. A. (1951). Familial differences in the proportion of abnormal haemoglobin present in the sickle-cell trait. *J. Clin. Invest.* **30**, 1120.

Neubauer, O. (1909). Ueber den Abbau der Aminosäuren im gesunden und kranken Organismus. *Dtsch. Archiv. klin. Med.* **95**, 211.

Neubauer, O. (1928). *Handb. norm. pathol. Physiol.* Berlin: Julius Springer.

Neubauer, O. & Falta, W. (1904). Ueber das Schicksal einiger aromatischer Säuren bei der Alkaptonurie. *Hoppe-Seyl. Z.* **42**, 81.

Neuberger, A., Rimington, C. & Wilson, J. M. G. (1947). Studies on alkaptonuria. 2. Investigations on a case of human alkaptonuria. *Biochem. J.* **41**, 438.

Parkes Weber, F. (1931). A glycosuric family without hyperglycaemia. *Lancet*, **2**, 71.

Pauling, L., Itano, H. A., Singer, S. J. & Wells, I. C. (1949). Sickle-cell anaemia, a molecular disease. *Science*, **110**, 543.

Penrose, L. S. (1951). Measurement of pleiotropic effects in phenylketonuria. *Ann. Eugen., Lond.*, **16**, 134.

Penrose, L. S. & Quastel, J. H. (1937). Metabolic studies in phenylketonuria. *Biochem. J.* **31**, 266.

Perutz, M. F., Liquori, A. M. & Eirich, F. (1951). X-ray and solubility studies of the haemoglobin of sickle-cell anaemia patients. *Nature, Lond.*, **167**, 929.

Perutz, M. F. & Mitchison, J. M. (1950). State of haemoglobin in sickle-cell anaemia. *Nature, Lond.*, **166**, 677.

Pieter, H. (1925). Une famille d'alcaptonuriques. *Pr. méd.* **33**, 1310.

Pomeranz, M. M., Friedmann, L. J. & Tunick, I. S. (1941). Roentgen findings in alkaptonuric ochronosis. *Radiology*, **37**, 295.

Prescott, B. A., Borek, E., Brecher, A. & Waelsch, H. (1949). Studies on oligophrenia phenylpyruvica. 1. Microbiological determination of L- and D-phenylalanine and of phenyllactic acid. *J. Biol. Chem.* **181**, 273.

PUTKONEN, T. (1930). Ueber die gruppenspezifischen eigenschaften verschiedener Korperflussigkeiten. *Acta Soc. Med. 'Duodecim'*, A, **14**, no. 2.

RACE, R. R. & SANGER, R. (1950). *Blood Groups in Man.* Oxford: Blackwell.

RICH, A. (1952). Studies on the haemoglobin of Cooley's anaemia and Cooley's trait. *Proc. Nat. Acad. Sci., Wash.*, **38**, 187.

ROSE, W. C., HAINES, W. J., JOHNSON, J. E. & WARNER, D. T. (1943). Further experiments on the role of the amino-acids in human nutrition. *J. Biol. Chem.* **148**, 457.

RYNBERGEN, H. J., CHAMBERS, W. H. & BLATHERWICK, N. R. (1941). Respiratory metabolism in fructosuria. *J. Nutrit.* **21**, 553.

SACHS, B., STERNFELD, L. & KRAUS, G. (1942). Essential fructosuria. Its pathophysiology. *Amer. J. Dis. Child.* **63**, 252.

SCHIFF, F. (1927). Ueber den serologischen Nachweis der Blutgruppeneigenschaft O. *Klin. Wschr.* **6**, 303.

SCHIFF, F. & SASAKI, H. (1932). Der Ausscheidungstypus, ein auf serologischen Wege nachweisbares Mendelndes Merkmal. *Klin. Wschr.* **34**, 1426.

SCHLESINGER, W. (1903). Zur Klinik und Pathogenese des Lävulosediabetes. *Arch. Exp. Path. Pharmak.* **50**, 273.

SCHROEDER, W. A., KAY, L. M. & WELLS, I. C. (1950). Amino-acid composition of haemoglobins of normal negroes and sickle-cell anaemics. *J. Biol. Chem.* **187**, 221.

SCUDAMORE, C. (1823). *A Treatise on the Nature and Cure of Gout and Gravel*, 4th ed. London.

SHANNON, J. A. (1939). Renal tubular excretion. *Physiol. Rev.* **19**, 63.

SHERMAN, I. J. (1940). The sickling phenomenon, with special reference to the differentiation of sickle-cell anaemia from the sickle-cell trait. *Johns Hopk. Hosp. Bull.* **67**, 309.

DE SILVA, C. C. & THANABALASUNDARAM, R. S. (1951). Congenital afibrinogenaemia. *Brit. Med. J.* **2**, 86.

SILVER, S. & REINER, M. (1934). Essential fructosuria. Report of three cases with metabolic studies. *Arch. Intern. Med.* **54**, 412.

SILVESTRONI, E. & BIANCO, I. (1952). Genetic aspects of sickle-cell anaemia and microdrepanocytic disease. *Blood*, **7**, 429.

SINGER, K. & CHERNOFF, A. I. (1952). Studies on abnormal haemoglobins. III. The interrelationship of type S (sickle cell) haemoglobin and type F (alkali resistant) haemoglobin in sickle-cell anaemia. *Blood*, **7**, 47.

SINGER, K., CHERNOFF, A. I. & SINGER, L. (1951*a*). Studies on abnormal haemoglobins. I. Their demonstration in sickle-cell anaemia and other haematological disorders by means of alkali denaturation. *Blood*, **6**, 413.

SINGER, K., CHERNOFF, A. I. & SINGER, L. (1951*b*). Studies on abnormal haemoglobins. II. Their identification by means of fractional denaturation. *Blood*, **6**, 429.

SINGER, K., ROBIN, S., KING, J. C. & JEFFERSON, R. N. (1948). Life span of sickle cell and pathogenesis of sickle-cell anaemia. *J. Lab. Clin. Med.* **33**, 975.

VAN SLYKE, D. D., HILLER, A., WEISIGER, J. R. & CRUZ, W. O. (1946). The determination of carbon monoxide in blood and of total and active haemoglobin by carbon monoxide capacity. Inactive haemoglobin and methaemoglobin contents of normal human blood. *J. Biol. Chem.* **166**, 121.

SMYTH, C. J., COTTERMAN, C. W. & FREYBERG, R. H. (1948). The genetics of gout and hyperuricemia—an analysis of nineteen families. *J. Clin. Invest.* **27**, 749.

SNYDER, L. H. (1932). The inheritance of taste deficiency in man. *Ohio J. Sci.* **32**, 436.

SOISALO, P. (1933). Ueber die renale Glykosurie. *Acta Soc. Med. 'Duodecim'*, **14**, 1.

STECHER, R. M., HIRSH, A. H. & SOLOMONS, W. M. (1949). The heredity of gout and its relationships to familial hyperuricemia. *Ann. Intern. Med.* **31**, 595.

STOWERS, J. & DENT, C. E. (1947). Studies on the mechanism of the Fanconi syndrome. *Quart. J. Med.*, N.S., **16**, 275.

STURGEON, P., ITANO, H. A. & VALENTINE, W. N. (1952). Chronic haemolytic anaemia associated with thalassaemia and sickling traits. *Blood*, **7**, 350.

TALBOTT, J. H. (1940). Serum urate in relatives of gouty patients. *J. Clin. Invest.* **19**, 645.

TALIAFERO, W. H. & HUCK, J. G. (1923). The inheritance of sickle-cell anaemia in man. *Genetics*, **8**, 594.

THOMSEN, O., FRIEDENREICH, V. & WORSAAE, E. (1930). Existenz zweier neuer Blutgruppen; auch ein Beitrag zur Beleuchtung sogenannter Untergruppen. *Acta path. microbiol. scand.* **7**, 157.

TOENISSEN, A. (1922). Ueber die Vererbung der Alkaptonurie des Menschen. *Z. indukt. Abstamm.- u. VererLehre*, **29**, 26.

UDENFRIEND, S. & COOPER, J. R. (1952). The enzymatic conversion of phenylalanine to tyrosine. *J. Biol. Chem.* **194**, 503.

WATSON, J., STAHMAN, A. W. & BILELLO, F. P. (1948). Significance of paucity of sickle cells in newborn Negro infants. *Amer. J. Med. Sci.* **215**, 419.

Weinberg, W. (1908). Ueber den Nachweis der Vererbung beim Menschen. *Jh. Ver. vaterl. Naturk. Württemb.* **64**, 369.

Weinberg, W. (1927). Mathematische Grundlagen der Probandenmethode. *Z. indukt. Abstamm.- u. VererbLehre*, **48**, 179.

Wells, I. C. & Itano, H. A. (1951). Ratio of sickle-cell anaemia haemoglobin to normal haemoglobin in sicklemics. *J. Biol. Chem.* **188**, 65.

Wiener, A. S. (1943). *Blood Groups and Transfusion.* Illinois: C. C. Thomas.

Wiener, A. S. (1951). Heredity of the M–N types. Analysis of twenty years' work. *Amer. J. Hum. Genet.* **3**, 179.

Wilson, S. A. K. (1912). Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. *Brain*, **34**, 295.

Wolfson, Q. W., Hunt, H. D., Levine, R., Guterman, H. S., Cohn, C., Rosenberg, E. F., Huddleston, B. & Kadota, K. (1949*a*). The transport and excretion of uric acid in man. V. A sex difference in urate metabolism. *J. Clin. Endocrin.* **9**, 749.

Wolfson, Q. W., Guterman, H. S., Levine, R., Cohn, C., Hunt, H. D., Rosenberg, E. F., Kadota, K. & Turner, L. (1949*b*). An endocrine finding apparently characteristic of gout. *J. Clin. Endocrin.* **9**, 497.

Wolkow, M. & Baumann, E. (1891). Ueber das Wesen der Alkaptonurie. *Hoppe-Seyl. Z.* **15**, 228.

Wollaston, W. H. (1810). On cystic oxide, a new species of urinary calculus. *Phil. Trans. Roy. Soc.* p. 223.

Womack, M. & Rose, W. C. (1934). Feeding experiments with mixtures of highly purified amino-acids. VI. The relation of phenylalanine and tyrosine to growth. *J. Biol. Chem.* **107**, 449.

Woolf, L. I. (1951). Excretion of conjugated phenylacetic acid in phenylketonuria. *Biochem. J.* **49**, ix.

EUGENICS LABORATORY PUBLICATIONS

LECTURE SERIES

I. The Scope and Importance to the State of the Science of National Eugenics. By KARL PEARSON, F.R.S. Third Edition. 1911. Price 1*s.* 6*d.* net.

II. The Groundwork of Eugenics. By KARL PEARSON, F.R.S. Second Edition. 1912. Price 1*s.* 6*d.* net.

III. The Relative Strength of Nurture and Nature. Much enlarged Second Edition. PART I. The Relative Strength of Nurture and Nature. By ETHEL M. ELDERTON. PART II. Some Recent Misinterpretations of the Problem of Nurture and Nature. By KARL PEARSON, F.R.S. 1915. Price 2*s.* 6*d.* net.

IV. On the Marriage of First Cousins. By ETHEL M. ELDERTON. 1911. Price 1*s.* 6*d.* net.

V. The Problem of Practical Eugenics. By KARL PEARSON, F.R.S. 1912. Price 1*s.* 6*d.* net.

VI. Nature and Nurture, the Problem of the Future. By KARL PEARSON, F.R.S. Second Edition. 1913. Price 1*s.* 6*d.* net.

VII. The Academic Aspect of the Science of National Eugenics. By KARL PEARSON, F.R.S. 1911. Price 3*s.* net. Sold only with complete sets.

VIII. Tuberculosis, Heredity and Environment. By KARL PEARSON, F.R.S. 1912. Price 1*s.* 6*d.* net.

IX. Darwinism, Medical Progress and Eugenics. The Cavendish Lecture. 1912. By KARL PEARSON, F.R.S. Price 1*s.* 6*d.* net.

X. The Handicapping of the First-born. By KARL PEARSON, F.R.S. 1914. Price 2*s.* 6*d.* net.

XI. National Life from the Standpoint of Science. (Third Issue.) 1905. By KARL PEARSON, F.R.S. Price 1*s.* 6*d.* net.

XII. The Function of Science in the Modern State. By KARL PEARSON, F.R.S. 1919. Price 2*s.* net.

XIII. Sidelights on the Evolution of Man. By KARL PEARSON, F.R.S. 1921. Price 3*s.* net.

XIV. The Right of the Unborn Child. By KARL PEARSON, F.R.S. 1927. Price 3*s.* net.

MEMOIR SERIES

IV. On the Measure of the Resemblance of First Cousins. By ETHEL M. ELDERTON, assisted by KARL PEARSON, F.R.S. 1907. Price 5*s.* net.

V. A First Study of the Inheritance of Vision and of the Relative Influence of Heredity and Environment on Sight. By AMY BARRINGTON and KARL PEARSON, F.R.S. 1909. Price 5*s.* net.

VII. On the Relationship of Condition of the Teeth in Children to Factors of Health and Home Environment. By E. C. RHODES, D.SC. 1921. Price 9*s.* net.

X. The Influence of Parental Alcoholism on the Physique and Intelligence of the Offspring. By ETHEL M. ELDERTON, assisted by KARL PEARSON, F.R.S. 1910. Price 6*s.* net.

XIII. A Second Study of the Influence of Parental Alcoholism on the Physique and Intelligence of the Offspring. By KARL PEARSON, F.R.S., and ETHEL M. ELDERTON. 1910. Price 6*s.* net.

XIV. A Preliminary Study of Extreme Alcoholism in Adults. By AMY BARRINGTON and KARL PEARSON, F.R.S., assisted by DAVID HERON, D.SC. 1910. Price 6*s.* net.

XVII. A Second Study of Extreme Alcoholism in Adults. With special reference to the Home-Office Inebriate Reformatory Data. By DAVID HERON, D.SC. 1912. Price 6*s.* net.

XVIII. On the Correlation of Fertility with Social Value. A Co-operative Study. 1913. Price 7*s.* 6*d.* net.

XIX–XX. Report on the English Birthrate. Part I. England, North of the Humber. By ETHEL M. ELDERTON, Galton Research Fellow. 1914. Price 9*s.* net.

XXXVI. Race Crossing in Man. The Analysis of Metrical Characters. By J. C. TREVOR, B.SC., M.A., PH.D. Price 12*s.* 6*d.* net.

XXXVII. An Introduction to Human Biochemical Genetics. By H. HARRIS, M.A., M.D. Price 15*s.* net.

Application should be made to the

CAMBRIDGE UNIVERSITY PRESS, BENTLEY HOUSE, LONDON, N.W.1

EUGENICS LABORATORY PUBLICATIONS

THE TREASURY OF HUMAN INHERITANCE

VOLUME I

Parts I and II. *Mem. Ser. VI.* Diabetes insipidus, Split-Foot, Polydactylism, Brachydactylism, Tuberculosis, Deaf-Mutism, and Legal Ability. Price 14*s.* net.

PART III. *Mem. Ser. IX.* Angioneurotic Oedema, Hermaphroditism, Deaf-Mutism, Insanity, Commercial Ability. Price 10*s.* net.

PART IV. *Mem. Ser. XI.* Cleft Palate, Hare-Lip, Deaf-Mutism, and Congenital Cataract. Price 10*s.* net.

PARTS V and VI. *Mem. Ser. XII.* Haemophilia. Price 15*s.* net.

PARTS VII and VIII. *Mem. Ser. XV.* Dwarfism. Price 15*s.* net.

Prefatory matter and indices to VOL. I. *Mem. Ser. XVI.* Price 5*s.* net.

VOLUME II

PART I. *Mem. Ser. XXI.* On Retinitis Pigmentosa and Allied Diseases, Congenital Stationary Night-Blindness and Glioma Retinae. Price 45*s.* net.

PART II. *Mem. Ser. XXIII.* Colour Blindness. Price 45*s.* net.

PART III. *Mem. Ser. XXIV.* Blue Sclerotics and Fragility of Bone. Price 36*s.* net.

PART IV. *Mem. Ser. XXVI.* Hereditary Optic Atrophy (Leber's Disease). Price 36*s.* net.

PART V. *Mem. Ser. XXVII.* On some Hereditary Structural Anomalies of the Eye, and on the Inheritance of Glaucoma. Price 36*s.* net.

Prefatory matter and indices to VOL. II. *Mem. Ser. XXVIII.* Price 9*s.* net.

VOLUME III

PART I. *Mem. Ser. XXII.* Hereditary Disorders of Bone Development. Price 45*s.* net.

VOLUME IV

Nervous Diseases and Muscular Dystrophies.

PART I. *Mem. Ser. XXIX.* Huntington's Chorea. By JULIA BELL, M.A., M.R.C.P. Price 10*s.* net.

PART II. *Mem. Ser. XXX.* On the Peroneal Type of Progressive Muscular Atrophy. By JULIA BELL, M.A., M.R.C.P. Price 12*s.* 6*d.* net.

PART III. *Mem. Ser. XXXI.* On Hereditary Ataxia and Spastic Paraplegia. By JULIA BELL, M.A., F.R.C.P. Assisted by E. ARNOLD CARMICHAEL, M.B., F.R.C.P. Price 17*s.* 6*d.* net.

PART IV. *Mem. Ser. XXXII.* On Pseudo-hypertrophic and Allied Types of Progressive Muscular Dystrophy. By JULIA BELL, M.A., F.R.C.P. Price 12*s.* 6*d.* net.

PART V. *Mem. Ser. XXXIII.* Dystrophia Myotonica and Allied Diseases. By JULIA BELL, M.A., F.R.C.P. With Clinical Notes by J. PURDON MARTIN, M.D., F.R.C.P. Price 21*s.* net.

Prefatory note and indices to VOLUME IV. *Mem. Ser. XXXIV.* Price 5*s.* net.

VOLUME V

Hereditary Digital Anomalies.

PART I. *Mem. Ser. XXXV.* On Brachydactyly and Symphalangism. By JULIA BELL, M.A., F.R.C.P. Price 12*s.* 6*d.* net.

PART II. *Mem. Ser. XXXVIII.* On Syndactyly and its Association with Polydactyly. By JULIA BELL, M.A., F.R.C.P. Price 10*s.* 6*d.* net.

VOLUME I *is obtainable at the price of* 52*s.* 6*d. net, and* VOLUME II *at the price of* 155*s. net.*
VOLUME IV *price* 59*s. net.*

Application should be made to the
CAMBRIDGE UNIVERSITY PRESS, BENTLEY HOUSE, LONDON, N.W.1